WORLD HEALTH ORGANIZATION

MONOGRAPH SERIES

No 22

PLAGUE

PLAGUE

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World Health Organization



WORLD HEALTH ORGANIZATION

PALAIS DES NATIONS

GENEVA

1954

A French edition of this monograph is in preparation

NOTE

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PRINTED IN SWITZERLAND

FIGURES

In reviewing the history of plague research from the discovery of *Pasteurella pestis* in 1894 to the present day one cannot fail to perceive that, although work in this field of applied science was carried on assiduously the whole time the greatest progress was made at the beginning and at the end of this sixty year period During the twenty years immediately following the isolation of the causative organism the work of such pioneers as Yersin Simond, Albrecht and Ghon together with the comprehensive studies of the Plague Research Commission in India laid a secure foundation for future investigations and it was also in this period that plague vaccination was introduced by Haffkine and thus the first milestone on the road to effective plague control was reached But it is only within the last decade that treatment with sulfonamides and antibiotics on the one hand and the application of potent insecticides—particularly DDT—on the other have rendered plague both a normally curable and a thoroughly controllable disease

The last publication in the English language to deal comprehensively with the plague problem was a manual compiled in 1936 by Wu Lien teh J W H Chun R Pollitzer and C Y Wu Appearing as it did before the spectacular advances in the treatment and control of the disease had begun this work while still appreciated as a reference book has become rather outmoded For this reason and because he was the only one of the four authors who had remained active in plague research the present writer was repeatedly urged to bring out a second edition However quite apart from the fact that the writer s work on plague and cholera control occupied the major part of his time both during and immediately after the second World War several considerations militated against a re edition of the 1936 manual In the first place the book was written primarily for workers in China and contains some sections of little interest for the general reader Secondly as already indicated the parts concerned with treatment and with the control of rats and fleas have become so obsolete as to be of historical rather than practical importance

Hence it became clear that it would be far better to publish an entirely new monograph dealing with the plague problem as it exists today than to attempt a revision of the 1936 manual

It was therefore decided that initially, a number of studies, dealing successively with all aspects of plague should be published in the *Bulletin of the World Health Organization* and that, eventually these individual studies should be grouped and reprinted in book form The first study was published in November 1951 the last in September 1953 Before publication in the present monograph the studies were carefully revised and brought up to date It is hoped that the final result will be of real assistance to workers engaged in plague research

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ACKNOWLEDGEMENTS

The writer s responsible task of compiling this monograph has been greatly alleviated through the advice and help generously given by many colleagues and friends Invidious as it is to single out some of those who have thus earned his gratitude the writer wishes to mention specifically Mr D H S Davis of the Plague Research Laboratory Union Department of Health Johannesburg Union of South Africa who provided much useful information on plague infected rodents and fleas in Africa Mr G H E Hopkins of the British Museum (Natural History) Zoological Museum Tring England whose assistance was of great value in connexion with the chapters on rodents and fleas Dr V B Link and Dr C O Mohr of the Communicable Disease Center Atlanta Ga USA who supplied additional data on plague in the United States of America Dr A Macchiavello formerly Expert Consultant on Plague at the Pan American Sanitary Bureau Washington D C USA who kindly made available unpublished lists of reservoirs and vectors of plague and furnished much valuable information on plague in South America Dr K F Meyer Director of the George Williams Hooper Foundation University of California Medical Center San Francisco USA who was indefatigable in giving his generous assistance in many ways Mr F G A M Smit Custodian of the Rothschild Collection of Siphonaptera at the Zoological Museum Tring who contributed an important annex—enriched by excellent drawings he had prepared himself—on the identification of fleas and Dr S S Sokhey formerly Director of the Haffkine Institute Bombay and Dr P M Wagle the present Director of the Institute who supplied most useful information on plague and its control in India

In addition to making the foregoing personal acknowledgements the writer would like to express his indebtedness to all who kindly contributed material for the illustrations to this monograph Some of these were provided through the courtesy of certain institutions namely

The George Williams Hooper Foundation University of California Medical Center San Francisco Calif USA (fig 5–9 15–23 and 32)

The Institut Pasteur Paris France (fig 11 and 12) and the Institut Pasteur Madagascar (fig 33 and 34)

The Wellcome Museum for Medical Science London England Dr G Robertson Capetown Union of South Africa and Colonel P V Karanchandani Madras India (fig 28–31)

The Haffkine Institute Bombay India (fig 24–26) and

Ciba Aktiengesellschaft Basle Switzerland (fig 1 2 and 10)

Others have been reproduced by kind permission of the editors from the following publications

Indian Journal of Medical Research 1939/40 27 325 326—fig 13 and 14 (by permission of the Indian Council of Medical Research)

Rat borne disease prevention and control 1949 pp 55 60 61 266 (United States Public Health Service Communicable Disease Center Atlanta Ga) —fig 27 36 37 and 39

Control of rats and mice 1948 pp 20 25 (University of California College of Agriculture Agricultural Extension Service Circular 142)—fig 35 and 38

Finally the author wishes to express his gratitude to Dr G Girard Chef du Service de la Peste Institut Pasteur Paris who besides rendering valuable help in other ways has undertaken the onerous task of translating the studies into French Thanks to his work the readership of the monograph will be greatly increased

Chapter 1

HISTORY AND PRESENT DISTRIBUTION OF PLAGUE

HISTORICAL SUMMARY

Dealing with the historical aspects of the plague problem in 1936, Wu Lien teh[160] maintained that the disease had been present since time immemorial in the areas within or near the Central Asiatic plateau which he considered as the original home of the infection He noted that some authors were inclined to place this in Central Africa but though the focus existing there was undoubtedly of very old standing Wu Lien teh was in agreement with the statement of Payne[111] that possibly if we could follow the history far enough back, we might find that the African was a colony of the Asiatic plague

Basing his statement upon the authority of Sticker[141] Wu Lien teh maintained that the first plague epidemic on actual record was the outbreak among the Philistines in 1320 B C which as described in the Bible (1 Samuel v and vi) was characterized by the appearance of emerods in their secret parts

As claimed by some recent writers this interpretation of the biblical text has become untenable Dealing with this subject in 1942 Neustatter[108] pointed out the interesting fact that the identity of the emerods with plague boils had been mentioned already in a marginal note to the revised version of the Bible appearing in 1885 that is at a time when people in Europe paid little if any attention to the subject of plague It seemed however that the 1885 compilers had followed the lead of the Bible commentator Thenius who had asserted the plague nature of the Philistine outbreak about 50 years earlier Thenius in his turn had probably been influenced by the writings of J J Scheuchzer (1672 1733) doctor of medicine and

professor of mathematics and physics at Zurich Switzerland, referring to the 1720 epidemic in Marseilles France

While not definitely committing himself as to the real nature of the biblical outbreak, Neustatter concluded that the version hemorrhoids, untenable as it is, comes nearer to the truth than plague boils Shrewsbury[136] and Girard[37] felt certain that the emerods were really haemorrhoids because they considered that the disease decimating the Philistines was bacillary dysentery However, this assumption was vigorously opposed by MacArthur[80] who asserted the plague nature of the Philistine outbreak on historio epidemiological and philological as well as on clinical grounds and, in a further note[81] also adduced evidence putting the great antiquity of the rat [*Rattus rattus*] in Palestine beyond question

A further generally accepted record testifying to the existence of plague in the West during the pre Christian era is contained in a fragment from the writings of Rufus, physician at Ephesus about A D 100 who noted the occurrence of fatal bubonic plague in Libya, Egypt, and Syria during and before his time apparently as far back as about the end of the third century B C (Wu Lien teh[160])

Whether this scanty information refers to occasional manifestations of the disease which remained localized or whether some of these outbreaks were episodes of an early pandemic it is impossible to decide It is certain that the first really satisfactory evidence regarding the prevalence of plague concerns a pandemic commencing *in the fifteenth year of the Emperor* Justinian s reign (A D 542) which was voluminously dealt with by contemporaneous writers In the opinion of most of these chroniclers, the pandemic had started at Pelusium in Lower Egypt, but probably this port served merely as the distributing centre of an infection derived from an endemic focus The contention of Evagrius that the plague had come from Ethiopia might suggest a Central African origin of the pandemic during Justinian s reign (Wu Lien teh[158])

Lasting for a period of fifty to sixty years, the pandemic gradually spread as one of the chroniclers put it, to the ends of the habitable world Usually seaports were invaded first, the infection then progressing inland and eventually involving even the most sequestered localities (Procopius quoted by Gibbon[33]) It was estimated at the time that the number of victims might have reached a total of one hundred million and Gibbon when scrutinizing the records of the contemporaneous writers, considered this figure as not wholly inadmissible Certainly the outbreak of plague in Justinian s time which, as deplored by the chronicler Warnefried depopulated towns, turned the country into a desert, and made the habitations of men to become the haunts of wild beasts was one of the worst calamities that ever befell mankind As Hirsch[51] points out it is possible that the simultaneous presence of other epidemic diseases partly accounted for this death toll but the contemporaneous records leave no

doubt that bubonic plague took the foremost part The question of whether or not commensal rodents played an important role in the causation of these bubonic epidemics is much debated In the opinion of many writers, because rats (*R rattus*) were not imported into Europe before the 12th century through the ships of the returning crusaders they were still absent at the time of the pandemic during Justinian s reign It is generally admitted that commensal mice were already abundant in the West at that period but as stressed by Shrewsbury[136] and Girard[37] usually these rodents though apt to become secondarily involved in the course of rat epizootics do not play an independent role in the causation of human outbreaks The same view was strenuously advocated by Tricot Royer[149] who came to the conclusion that

"interhuman transmission formerly played a considerable if not predominant role in the spread of epidemics and was often even the only factor concerned [a]

However it deserves attention that this opinion was not shared by MacArthur[80] who adduced evidence pointing to the early presence of rats in Europe

Though it is likely that plague outbreaks due to fresh importation of the infection if not to its local persistence continued to occur in the West as well as in the East the information actually available is usually unsatisfactory until once more the evil culminated in a pandemic which as Hirsch[1] put it

"arrested the attention of the chroniclers poets and physicians of these days and that interest was awakened by the enormous diffusion that it reached over the whole of the then known world by its victims reckoned in millions and by the shock to the framework of society which it brought with it and left behind it

While it is uncertain whether the pneumonic form of the disease played an important part in the plague outbreaks in Justinian s reign this type figured prominently in the pandemic of the 14th century which for not fully elucidated reasons (see Wu Lien teh[160]) became known under the name of the Black Death However rats being without doubt implicated in the perpetuation of the infection the bubonic form was also frequent or even preponderant as for instance in rural England (Greenwood[41])

As mentioned above it is possible that in the case of the pandemic in Justinian s time the infection had been derived from the Central African plague focus No doubt can exist that the Black Death originated in Central Asia Circumstantial evidence for this assumption was brought forward by Wu Lien teh[158] who showed that this pandemic was not restricted to Europe and the Near East but was rampant in India and China as well a spread of the infection from its original home in inner Asia southwards and eastwards as well as towards the west was therefore likely

[a] dans l'épidémisation ancienne la contamination interhumaine jouait un rôle considérable sinon prédominant et souvent même exclusif

Through a fortunate accident the present writer was able to obtain confirmation for this contention In a book entitled *The Nestorian Missionary Enterprise* by Stewart [140] a reference was found to the work of the Russian archeologist Chwolson near Issyk Kul Lake in the Semirechinsk district, an area now known to lie within the Central Asiatic plague focus Chwolson found in old Nestorian graveyards three memorial stones dating back to the years 1338 9, the inscriptions on which showed that the persons referred to had succumbed to plague It was, moreover, evident that during the period in question an exceptionally large number of burials had been made It is certain, therefore that plague was conspicuous in Central Asia a few years before the Crimean ports became infected (1346) and the disease was carried from there by ship to Europe

In Great Britain, from half to two thirds of the people are believed to have been killed by the Black Death Hecker's [50] estimate that 25 millions or one fourth of the population fell victim to the pandemic in Europe was therefore perhaps conservative Millions succumbed in Asia as well where, according to Gabriel de Mussis, an eyewitness of the outbreak in the Crimea

> so great was the mortality that Arabs Saracens and Greeks throughout the whole of the East gave themselves up to clamour

It was inevitable that in many places the infection introduced during the pandemic became firmly entrenched among the rats As a result, many countries in Europe continued to have frequent or even perennial epidemics during the centuries following the Black Death However as described by Wu Lien teh [160] in the course of the 17th century a decline set in, leading to the gradual disappearance of the disease first from western and then from eastern Europe until in 1841 Turkey the last stronghold of the pest, became free

The reasons for the cessation of plague in Europe have been the subject of much debate Great stress was laid by some authors upon the change in the rat population by which *R rattus* became largely superseded by the sewer-rat (*R norvegicus*) However, as shown in a classical study by Jorge,[65] the invasion of the latter rodent, taking place during the first half of the 18th century, occurred long after the retrogression of plague from the western part of the continent Some writers considered the progress of civilization which led to higher standards of cleanliness housing and sanitation a possible cause for the disappearance of the infection However, as pointed out by Wu Lien teh,[160] a lull during the period under consideration was conspicuous not only in Europe but in the Near East, India and China as well It seemed likely, therefore, that a natural decline of plague was responsible for the cessation of the outbreaks, rather than extrinsic factors which at best could have been of auxiliary importance only and became fully operative well after the disappearance of the evil from Europe

Though as discussed above autochthonous plague ceased to exist in Europe and many parts of the East, occasional outbreaks due to an importation of the infection from still active foci continued to occur Thus while France had become generally free by 1668, an epidemic, believed to have been due to importation from Syria, and sweeping away 50,000 people (Gibbon[33]) took place at Marseilles, France in 1720 the infection even spread over a great part of Provence but disappeared in 1722 Likewise while most parts of India seem to have become plague free at the end of the 17th century, outbreaks supposed by some to be due to importation from Persia, occurred during the period 1812 21 at Cutch, Gujarat, and Kattyawar, and in 1836 8 at Pali in Rajputana

Far more important than these outbursts was that, with its deep rooted tendency to become latent rather than to disappear altogether, plague continued to linger in quite a considerable number of endemic foci As stated by Wu Lien teh[158] the most important of these were situated in and around the Central Asiatic plateau in Russian Turkistan, Semirechinsk Chinese Turkistan Inner Mongolia, Outer Mongolia, and Transbaikalia in the foothills of the Himalayas in northern India in Kurdistan as well as in Central Africa and possibly also in parts of North Africa

It would seem that by the end of the 18th century plague outbreaks had become frequent in the north east of Burma and that inroads of the infection into the adjacent part of Yun nan Province in China took place As discussed by Wu Lien teh[160] during the first half of the 19th century the disease appears to have become established in the extreme west of Yun nan without however prevailing epidemically

Quite possibly under normal conditions the infection would have continued to smoulder in west Yun nan without spreading farther afield Most unfortunately however the equilibrium was upset by a rebellion of the Mohammedans commencing in 1855 for the suppression of which troops had to be sent in Their operations and possibly also movements of refugees provided suitable means for a propagation of the disease which was to prove disastrous not only for China but for many other parts of the world

Progressing in general by slow stages plague reached the provincial capital Yun nan fu (now Kunming) in 1866 and it took 28 years more before Canton and Hong Kong were reached in 1894 Rather surprisingly however the disease appeared at Pak hoi in Southern Kwang tung in 1867 already Serious doubts were entertained as to whether the invasion of this port could have been due to a long distance sprint of the infection from Yun nan However since it is difficult to see by what other route Pak hoi could have been reached one has to accept the explanation of Simpson [138]

" An epidemic of plague occurs in Yunnanfu in 1866 which decimates the population while they are in the midst of war and in 1867 Pakhoi one of the homes of returning troops from Yunnan is attacked

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It is depressing to reflect that the situation confronting the world when Hong Kong was invaded was in some respects even worse than that at the onset of the pandemic of Justinian's time, in Pelusium For while in A D 542 the means for transporting the infection were slow and comparatively inadequate, and the orbit within which it could spread was limited, in 1894 steamships and railways had replaced the small sailing craft and caravans of Justinian's day and the new as well as the old world were open to the inroads of plague It is true that progress in civilization and public health had made it impossible for the infection to gain a firm foothold in modern Europe, but in many other parts of the world ample fuel was available for its perpetuation and spread

To draw even in broad lines an adequate picture of the progress of plague since 1894 would be a task far beyond the scope of the present survey All that can be done and at the same time all that is really needed for the purposes of this monograph, is to come to a general appreciation of the situation in those areas where plague continues to exist or was recently present

In compiling the present survey, advantage has been taken of the reports on the prevalence of plague in recent years published by Stowman [14] in 1945 and by Kaul [69] in 1949, and for most African plague foci also of the results of an inquiry instituted by the WHO Expert Committee on Plague at its first session (1950) Valuable information on some of the plague foci in East Asia was found in a series of reports by Wilcocks,[156] while in the case of the Americas the studies of Moll & O'Leary [103] served as a useful guide

PRESENT DISTRIBUTION OF PLAGUE

Asia

(1) *China*

When trying to assess the present plague situation in China it is neces sary to consider

(*a*) The incidence of the disease at Pak hoi since 1867, and at Canton and Hong Kong since 1894 and the spread of infection into Kwang tung Province of which Canton is the capital and to which Hong Kong belongs geographically,

(*b*) The invasion of other provinces from Canton and Hong Kong by the sea route

(*c*) Outbreaks due to recent entry of the infection by inland routes ,

(*d*) The reappearance of plague in Yun nan Province

(*a*) *Kwang tung Province* Epidemics at Pak hoi, the first port which had been reached by plague continued to be frequent up to the year 1902

and again from 1910 to 1915 the apparent lull during the period 1903 9 being presumably due to mere lack of information

Perennial outbreaks of varying intensity continued to occur at Hong Kong until 1923 after that year sporadic cases were seen in 1928 and 1929 As maintained by Uttley [160] in a retrospective study on plague in Hong Kong the decline of the infection could not be ascribed to the sanitary improvements made because even before 1923 the severity of the outbreaks had diminished elsewhere in south China In fact as far as one is permitted to judge from incomplete figures epidemics had ceased to be perennial in Canton as early as 1916 with subsequent outbreaks in 1923 and 1925 (Wu Lien teh [169])

The invasion of Canton in 1894 led almost immediately to a contiguous spread of the infection into Kwang tung Province most parts of which became successively affected This period of expansion was followed by a gradual decline becoming manifest about the end of the first World War At present only some districts situated at or near the Lui chow Peninsula in the south of the province and east of Pak hoi continue to be involved but it would seem that the situation there has become worse recently 627 cases having been reported during the period January September 1950 as against 186 cases in 1949 Presumably plague also continues on the island of Hainan which was originally invaded in 1900 (Landauer [7])

(*b*) *Spread of the infection from Kwang tung to other coastal provinces* Amoy the principal port of south Fu kien became affected in 1894 soon after the appearance of the disease at Canton and Hong Kong In 1899 the infection made a long distance sprint to the port of Newchwang in south Manchuria whereas Foochow the capital of Fu kien was invaded in 1901

The disease continued to reappear at Newchwang and in its immediate hinterland for some years only The invasion of Amoy and Foochow on the contrary had serious and lasting consequences Plague not only continued to appear perennially in these two cities for a number of years but owing principally to the dense water borne traffic to smaller seaports as well as up the rivers most of the counties of Fu kien Province became successively affected Though the frequency and extent of the infection seemed to be subject to variation the recorded incidence of plague being low in certain years the situation during the decade from 1937 to 1946 was in general rather serious 4 764 cases in 35 counties were recorded in 1943 7 157 cases in 42 counties in 1946 in both years serious epidemics occurred in Foochow city From 1947 to 1949 there was a gradual decrease in the case incidence but the situation seems to have become worse again in 1950 when up to the end of September 988 cases were reported as against a total of 368 cases during 1949

Amoy is now quite free from infection Rat epizootics continue to occur at Foochow but it has become possible to prevent the appearance

of human plague through the systematic use of DDT and measures aiming at a reduction of the rodent population

Plague in Fu kien Province has become increasingly rural in character Though pneumonic manifestations are occasionally met with the bubonic form of the disease is preponderant Commensal rats alone appear to be responsible for the perpetuation of the infection with *Xenopsylla cheopis* as the sole practically important vector

It has to be added that owing to intensified traffic over hitherto little used routes during the second World War the two provinces of Che kiang and Kiang si, formerly quite plague free, became affected in 1940 and 1941 respectively While the latter province seems to have been free from the end of 1949, a slight incidence of the disease continued during 1950 in Che kiang where Wenchow remains the only major port on the China coast still suffering from human plague

(*c*) *Outbreaks due to recent entry of the infection by inland routes* Since China may be said to have some of the regions composing the Central Asiatic focus at her back door, it is not surprising to find that the country remained open to inroads of plague from these endemic areas

Mention must first be made in this connexion of north Manchuria which becoming infected by human agency from the wild rodent foci in Outer Mongolia and Transbaikalia suffered from pneumonic plague epidemics in 1910 1 and again in 1920 1 Though exerting a grievous toll in deaths these outbreaks because they did not involve the local rat population disappeared as rapidly as they had come once the climatic conditions ceased to favour spread of the infection from man to man

In 1917 8 pneumonic plague apparently derived from a wild rodent focus in the Ordos country of Inner Mongolia (now Suiyuan) invaded Chahar and Shan si and even spread to a slight extent into Chihli, Shan tung An whei and Kiang su Provinces, the number of victims amounting to about 16 000 As described by Wu Lien teh [160] further outbreaks some of them mainly or solely bubonic in character continued to occur in the north west, infection being derived either from the Ordos Country or from endemic foci which had become established in Shen si In 1931 there was an ominous spread of the infection in Shan si and Shen si where, it was claimed, at least 20,000 people succumbed to mainly bubonic affections To judge from incomplete information, a decline seems to have set in since A mainly pneumonic outbreak with a case incidence of 485 started at the end of 1941 in Suiyuan and spread in 1942 also to Ningsia Shen si and Shan si (Fan [6]) The presence of an epidemic which originally bubonic in character later assumed pneumonic features was reported in 1949 from Chahar where 69 cases with 66 deaths occurred from July to November in 10 villages However since the northern part of the province was stated to have been involved it is possible that the infection was derived from the foci in south Manchuria or Jehol dealt with below

To trace the origin of the plague outbreaks proved to exist since 1927 in south west Manchuria is difficult Weighing the scanty available evidence Wu Lien teh [160] assumed that the infection the history of which could be traced back to about 1917 originated from a wild rodent focus in Inner Mongolia possibly in Chahar which as noted above had been involved in the 1917 8 outbreak However no doubt can exist that about the time when the presence of plague was confirmed in south west Manchuria epizootics existed among the commensal rats of the affected localities especially among *R norvegicus* (Hsiao) and *X cheopis* served as vector

The affected area at first restricted to the Tunghao region in the west gradually extended Even north Manchuria which had been free from plague since 1921 became involved in the 1946 outbreak while in 1947 the last year for which information is available 200 fatal cases were reported from Kirin Province in the east of south Manchuria

Jehol where an endemic focus existed at the close of the 19th century, again reported an outbreak in 1933 which was probably due to a fresh importation from south Manchuria To judge from the scanty information recently available the plague situation in Jehol became once more serious in 1946 when 15 000 cases seem to have been recorded The case incidence in 1947 when an energetic anti plague campaign was conducted seems to have amounted to about 2 000 Only a few dozen cases were recorded in 1948

While the epidemics in south Manchuria were preponderantly bubonic in character occasional pneumonic manifestations were met with An outbreak of this type (39 cases with 3 recoveries) in 1946 was described by Tieh et al [116]

(*d*) *Reappearance of plague in Yun nan Province* As in the case of the historic plague invasion of Yun nan dealt with above it is impossible to state exactly when the recent re entry of the infection into the province commenced Presumably now as then the manifest appearance of the disease had been preceded by unnoticed invasions of villages just across the Burma border It is certain that as the result of a serious outbreak at Nam Kham Burma in 1939 the infection not only reappeared early in 1940 in that area but also spread across the Shweli River into Chinese territory (UNRRA Report [149]) where however the Mungmao district alone became involved

While no information is available for 1941 and 1942 plague was again observed in the autumn of 1943 in a locality about two days walk from the Burma border In 1944 the disease not only assumed epidemic proportions in that area but also appeared in three other districts of Yun nan a total case incidence of 542 with 247 deaths being recorded

A further extension of the affected area took place in 1946 when the infection having crossed the Salween River reached Paoshan the principal city of west Yun nan The situation was not much changed in 1947 and

there seems to have been less plague in 1948 However, according to information received in 1949, the infection, having crossed the Me kong River, appeared in Ta-li At the same time plague was reported from three hitherto unaffected counties and a traveller from Paoshan was found to be suffering from the disease upon arrival at Kunming The total plague incidence in 1949 was 283 cases with 92 deaths as against 168 cases with 36 deaths in 1948

As will be gathered from the account given above, rat caused plague is entrenched in parts of south China as well as in the north west of the country and recently perennial outbreaks of the same type occurred in Yun nan as well On the other hand, plague has been either altogether absent from the central provinces or the infection, if imported upon rare occasions, has failed to establish itself

It should be noted in the latter connexion that plague was introduced into Shanghai in 1908 but though infected rats continued to be found every year until 1916 no widespread epizootics resulted and human cases never became numerous From 1917 onwards rat falls were few and far between or altogether absent and Shanghai has been quite free from plague since 1927

Likewise, when plague, believed to have been introduced by bacterial warfare, appeared at Chang teh, Hu nan Province, in 1941 (King,[70] Fan [6]) no permanent harm resulted even though in the following year a rat epizootic was rampant in that town and almost 100 human cases occurred The infection disappeared early in 1943 and since then Hu nan has been as free from plague as it was before the end of 1941

The absence of plague from central China or its failure to establish itself cannot be ascribed to a paucity of commensal rats or of *X cheopis*, both of which abound everywhere Likewise as confirmed by the observations in Hu nan, there is no reason to assume that plague resistant rat strains prevail in central China Probably however, peculiarities of the seasonal incidence of *X cheopis* go a long way to explain the freedom of central China As pointed out by Wu,[15] the incidence of that flea in Shanghai was low during the months of the plague season in south China, which commences in early spring He admitted that the autumnal incidence of the disease in the north exhibited a dangerous coincidence with the *cheopis* season in Shanghai but pointed out that under the existing conditions the southern plague foci alone were potentially dangerous for an importation of the infection into Shanghai

(2) *Burma*

Though as discussed above the historic as well as the recent invasion of Yun nan could be traced back to Burma, it would be rash to assert

FIG 1 INCISION OF A BUBO



Woodcut taken from "Item ein fast kostlicher Spruch von der Pestilenz" by Hans Folz, Nuremberg 1482

that permanent endemic foci comparable in standing to those in Central Asia exist in the latter country On the contrary it seems more likely that there as well as in west Yun nan prolonged periods during which rat epizootics continued and epidemics were consequently frequent alternated with quiescent periods In conformity with this concept Kaul[69] considered an importation of plague into Rangoon in 1905 as responsible for the present wave of infection which led to the establishment of endemic foci in Meiktila (especially the town of Mahlaing) Pyawbwe town and the northern Shan States near the Yun nan border (Wilcocks[1 6]) Though country districts as well as towns were involved according to Wilcocks 70% of the plague deaths were recorded in Rangoon and in the towns on the main lines of communication by river or rail

there seems to have been less plague in 1948 However, according to information received in 1949 the infection, having crossed the Me kong River, appeared in Ta li At the same time plague was reported from three hitherto unaffected counties and a traveller from Paoshan was found to be suffering from the disease upon arrival at Kunming The total plague incidence in 1949 was 283 cases with 92 deaths as against 166 cases with 36 deaths in 1948

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The incidence of the disease which had increased to 3,517 cases with 2 743 deaths in 1946, decreased until 1950 but then rose again as shown by the following figures

Year	*Cases*	*Deaths*
1947	1 518	1 192
1948	1 479	1 099
1949	799	641
1950	637	442
1951	900	583
1952	1 029	646

The usual plague season in Burma falls into the period November to April but in Lower Burma there may be a secondary rise in July (Wilcocks [156]) The bubonic type is preponderant but occasional pneumonic epidemics have been recorded, recently by Wynne Griffith [161] at Rangoon

Wilcocks [156] considered *R concolor* and *R rattus* of main importance in the perpetuation of plague in Burma the former was more common *R norvegicus* dwelling in fields and sewers seemed less dangerous Harrison & Woodville [47] found during a recent survey in Rangoon *R exulans concolor* most frequent (45%), followed by *Bandicota bengalensis* (31%) *R rattus* formed only 8% of the total, *R norvegicus* 6% However, plague infection was found only in two *R rattus* and one bandicoot

In some parts of Burma *Xenopsylla astia* was more commonly found than *X cheopis*, but the latter was responsible for most instances of human infection (Wilcocks [156])

(3) *Indochina*

Dr Robert in a valuable report on the plague situation in Indochina presented to the second session of the Joint *OIHP/WHO Study Group on* Plague stated that plague was endemic in the following localities

(*a*) Cochin China (South Viet nam) principally in Saigon/Cho lon the hotbed of the infection being located in the precincts round the central market of Saigon which had been invaded from Canton and Hong Kong *in 1906*

(*b*) Cambodia, especially Pnom Penh where plague probably imported from Saigon had become established in 1907

(*c*) Phan thiet/Phan Rang region on the south Annam Coast infected by ship from Saigon in 1908 ,

(*d*) Lang Bian plateau of south Indochina where the disease imported with rice cargoes from Cho lon, possibly also from Phan Rang became established in 1943 or 1944

The recent incidence of plague in the country was

Year	Cases	Deaths
1946	52	24
1947	90	40
1948	355	105
1949	113	55
1950 *	142	33
1951 *	119	39
1952 *	54	14

* Cambodia and Viet nam

While the bubonic type was most frequently met with, occasional epidemics with pneumonic features were observed (Wilcocks [1 6] Robert communication to WHO)

R rattus played in general the most dangerous role with *X cheopis* as the usual vector Most interestingly however Hervaux & Toumanoff, [3] investigating a limited outbreak at Saigon in 1943 (42 cases) proved the existence of an epizootic among domestic mice These animals while not harbouring any *Leptopsylla segnis* were more heavily *cheopis* infested than the rats Only one specimen of the latter (*R norvegicus*) was found infected

(4) *Thailand*

Plague probably imported by the sea route from China (Bangxang [3]) appeared at Bangkok in 1904 and it would seem that the inland town of Korat became infected in the same year

Though persisting in both places and eventually also involving other localities the infection seems to have caused comparatively little havoc in Thailand According to Wilcocks [1 6] a total of 1 722 cases occurred at Bangkok from 1905 to 1922 At Korat a severe outbreak (586 cases with 580 deaths) took place in 1917 Preventive measures including house improvement were then instituted and only 248 cases with 199 deaths were recorded from 1918 to 1934 Plague began to decline in the country in general from 1929 onwards and the disease seems to have been absent during the period from 1935 to 1937 (Bangxang [3]) However the infection reappeared towards the close of 1938 in the north western province of Tak and as described by Park [109] spread first eastwards and then to the south Bangkok became re infected early in 1940 but only a few cases were reported there and the port seems to have remained free from plague since that year

Korat became once more infected in 1942 since when cases or small groups of cases have been noted every year The recent incidence of

plague in Thailand may be gathered from the following

Year	Thailand Cases	Thailand Deaths	Korat Cases	Korat Deaths
1944	57	29	13	7
1945	107	46	19	2
1946	72	33	4	2
1947	71	23	4	1
1948	122	36	8	2
1949	176	64	1	1
1950	57	10	16	2
1951	5	1	(January November)	
1952	9	4		

Dealing with the seasonal occurrence of the disease Bangxang[3] stated that the case incidence began to increase in September and reached its highest point in February and March The disease was at a low level from May to September, particularly during the wet months of June and July Human plague was almost invariably of the bubonic type, but Bangxang[3] referred to one outbreak with pneumonic features described by Braddock in 1912

As maintained by Park[109] *R rattus alexandrinus* was principally involved , it formed over 60% of the Bangkok rats as against 14% *R norvegicus* Infected shrews were found and might, as Bangxang[3] believed, play a role in the propagation of the infection As everywhere in the Far East, *X cheopis* was the principal vector

(5) *Java*

Plague appeared in Java in 1911 but it is likely that the introduction of the infection, ascribed to the importation of rice cargoes into the port of Surabaya, went back to the previous year (November 1910) The interior of the eastern part of the island soon became invaded While it became possible to terminate the outbreaks there through wholesale rat proofing of the houses the infection began to move westwards successively affecting middle and western Java During the period 1920 7 from 8 000 to 10 000 deaths occurred annually A decrease of the infection rate was noted from 1928 to 1931 but then the incidence rose again, reaching a maximum of 23 267 cases with 23 239 deaths in 1934 Then, as shown below, a gradual decline set in which was ascribed to large scale inoculation campaigns with Otten s live vaccine

Year	Cases	Yea	Cases
1935	13 022	1939	1 558
1936	6 227	1940	312
1937	3 834	1941	550
1938	2 107	1942	339

Little information is available for the period during the second World War van Loghem[8] maintained that generally speaking the situation

was not alarming However though the case incidence reported for the period 1945 7 was low incomplete figures for 1948 and 1949 indicated a disquieting increase of the infection rate (3 422 cases with 3 365 deaths and 874 cases with 844 deaths respectively) the residency of Jogjakarta in central Java being most heavily affected

The recent incidence of plague in Java is shown below

	1950	*1951*		*1952*	
	Case	*Cases*	*Deaths*	*Cases*	*Deaths*
Eastern Java	8	310	221	52	13
Middle Java	3 450	4 292	1 993	1 524	820
Western Java	129	549	52	155	6

Incomplete

It will be noted that the plague situation in eastern Java was not serious In fact this part of the island seemed to be free from the infection during the second half of 1952 On the contrary middle Java particularly the residencies of Jogjakarta and Surakarta were seriously involved Plague caused less havoc in western Java but there can be no doubt that there as well as in middle Java endemic foci exist

As pointed out by Park[110] in Java where the temperature remains fairly uniform throughout the year no pronounced seasonal incidence of the disease was to be expected Still the plague mortality showed a tendency to increase during the third quarter of each year reaching its maximum in December and January then a decrease set in which lasted until July

Human plague in Java was mainly bubonic but cases with pneumonic features accounted for 6% 8% of the total incidence of the disease and outbreaks of pneumonic plague usually claiming 2 10 victims only were met with (Wu Lien teh[159] Wilcocks[156])

As summarized by Wilcocks[156] the brown Malayan house rat *R rattus diardi R concolor* inland and to a very small extent *R norvegicus* were implicated in the plague outbreaks in Java the first mentioned rodent being considered as the chief culprit *X cheopis* was the principal vector

(6) *India*

In a discussion on the endemicity of plague in India Sharif[13] postulated that *the infection is at present entrenched in the following foci*

(*a*) Three endemic centres situated near the foot of the Himalayas which though occasionally appearing to have been independent of one another possibly form part of a big sub Himalayan focus These centres were responsible for plague outbreaks in east Punjab the United Provinces and districts of Bihar north of the Ganges

(*b*) One focus in central India comprising the watersheds of the Vindhya Bhanrer and Maikal ranges and the Mahadeo hills

(c) Three centres in southern India situated respectively in the watersheds of the Western Ghats in Bombay State and Mysore , in watersheds located in the districts of Salem, Coimbatore Nilgiri, and Madura , in the Hyder abad State All three foci have been very active lately

There can be no doubt that the endemic centres in southern India became established after the city of Bombay had become infected in 1896 As stated by Sharif,[135] the first of them (Bombay and Mysore States) had been reached by plague in 1898 Nilgiri became involved in 1903 (George & Timothy [3]) The infection spread in 1898 from the then Bombay Presidency into the adjacent parts of Hyderabad State, but the situation there became serious in 1911 only when Hyderabad city became affected (Rao [1 0])

It seems possible on the other hand that the endemic foci in the Hima layas are partly of long standing Endemicity has been known to exist in the districts of Gharwal and Kumaon since 1823, but as assumed by Gill,[36] the infection persisting there was perhaps " a relic of the great pestilence in the 17th century Though these areas which are situated within the region involved at present, are known to have been responsible for plague outbreaks up to the year 1877 only latent infection might have continued to persist in some part of the Himalayan foothills to become active again early in the present century

The mortality from plague in India from 1898 to 1948 may be summarized as in table I

TABLE I MORTALITY FROM PLAGUE IN INDIA DURING THE PERIOD 1898 1948 a

Period	Total deaths from plague	Annual average	Deaths during each period expressed as percentage of total deaths 1898 1948
1898 1908	6 032 693	548 427	47 88
1909 1918	4 221 5[illegible]8	422 153	33 51
1919 1928	1 702 718	170 272	13 [illegible]2
1929 1938	422 880	42 288	3 36
1939 1948	217 970	21 797	1 73
Total 1898 1948	12 97 789	[illegible]47 015	100 00

a After Bhore Kaul

It will be noted that almost half of the total plague deaths in India occurred within the period 1898 1908 and more than three quarters up to 1919 Nevertheless as shown by the annual figures for the period 1939 52, set forth in table II, the recent plague situation in India was not as reassuring as it might seem at first glance The condition was quite favourable in 1942

TABLE II ANNUAL MORTALITY FROM PLAGUE IN INDIA DURING THE PERIOD 1939 52

Year	Total plague mortality	Bihar	Bombay State	Central Provinces (Madhya Pradesh)	Madras	Punjab	United Provinces (Uttar Pradesh)	Other areas	Hyderabad State[a]	Mysore State[a]
1939	6 57	1 933	1 4 ?	8?	3 4	—	21 662	9	6	352
19 0	19 97	1 040	5 5/3	3	1 169	—	11 / 5	9	7 00	2 5 3
1941	11 9	1 9	5 311	761	1 6	—	4 03	2	2 713	417
194	105 7	10	6 0	1 9	701	—	8 953	6	657	3 7/6
1943	13 5 8	6	715	144	4 8 5	1	7 6	11	1 498	3
19 4	21	834	514	910	1 738	61	15 4 4	14	5 63	3 7
1 5	0 1	1 5 3	11 /79	75	1 644	203	14 024		6 631	8 016
19	93	8 6 3	3 405	189	2 54	45	18 206	9	4 0 6	2 891
19 7	41 45	6 8	1 9	44	3 078	1 704	26 1 6	8	1 /91	1 0
19	9 57	90	855	2 4 6	9/8	196	4 384	16	811	1 12
9 3	57	6		2 60	151	27	2 904	/6	2 103	9
1 50	6 1	55	98	904	42	[b]	0/3	204	719	5
19 1	1 841	3		4 5	60	—	1 276	0	9	54
19	1 007	0	1	457	9	—	04	6	1	272
Total	3 6	894	41	16 40/	18 7 9	2 640	138 882	443	40 69	33 9/2

[a] Not included in totals [b] East Punjab only

but afterwards became worse once more the annual incidence rates for 1945 1946 and 1947 being increasingly in excess of that recorded in 1939 Kaul[6] assumed that the war with Japan was at least partly responsible for this recrudescence because other health data available in India at the time also showed a turn for the worse However the acute food scarcity existing in 1946 and 1947 in certain parts of the country which necessitated large scale movements of grain from central collecting stations to various provinces might have facilitated the dissemination of plague and a similar influence was probably exerted by the movements of large groups of people in connexion with the partition of India in August 1947 Since these unfavourable conditions were of a temporary nature it would be tempting to ascribe the considerably reduced incidence of the disease in 1948 and 1949 to an improvement of the general situation It is however disquieting to note that the 1950 plague mortality in the Central Provinces (now Madhya Pradesh) was markedly in excess of the figures reported for the three previous years Still the plague mortality there as well as in India in general was considerably lower in 1951 and 1952

Generally speaking in India as well as in China plague is now a rural rather than an urban problem It should be noted in this connexion that Bombay city where plague had been rampant up to 1923 and perennial manifestations had continued to exist until 1934, is now almost free In Calcutta, which apparently became affected in 1895 when suspicious cases were noted among a group of soldiers recently arrived from Hong Kong (Rao [119]) and where the infection persisted until 1925 considerable outbreaks due possibly to an importation from Bihar (Greval [41]), took place in 1948 and 1949 Only two autochthonous cases were recorded in 1950 but their number rose to 16 in 1951, and to 54 in 1952

Dealing with the seasonal incidence of the disease in his classical study Twenty years of plague in India', White [155] maintained that

"epidemics normally attain their maximum severity in Bombay in October in the Central Provinces in February in the United Provinces and Bihar in March and in the Punjab in April In the remaining provinces taken together March is the month of maximum mortality

While the bubonic type of the disease is by far the most frequent in India small outbreaks of pneumonic plague have been observed occasionally, recently by Seal [133] and Seal & Prasad [131] at Calcutta and Gaya (Bihar)

So far no evidence has been found that wild rodents played a role in the causation of plague outbreaks in India, the common species of com mensal rats forming the usual reservoir of the infection However as will be discussed later the bandicoots which are apt to come or even live near man also deserve some attention The Indian plague flea *X cheopis* is the typical vector of the infection

(7) *Western Asia (Iran, Iraq Turkey in Asia and Syria)*

A joint consideration of the plague regions in Western Asia seems justified in so far as according to Tholozan [141] and Payne [11] the mountains of Kurdistan were the chief endemic centre for a large area comprising Persian Kurdistan and adjacent parts of Persia, Turkish Kurdistan and parts of Mesopotamia Payne added that from this area plague extended

to Northern Persia on the shores of the Caspian (Resht) in 1877 to Baku on the western and Astrakhan on the northern shore of that sea and up the Volga to the village of Vetlianka and its neighbourhood in 1877 79

Presumably the persistence of the infection in the interior of western Asia was in part responsible for past plague invasions of Syria and Palestine, but it must be noted that these countries were open to inroads of the pest by the sea route as well

Findings made by Baltazard et al [°] after two limited outbreaks of pneumonic plague had been observed in the south of Iranian Kurdistan in October November 1947 have added much to the scanty information hitherto available in regard to the western Asiatic focus Baltazard and

his colleagues were able to prove the existence of plague in three species of *Meriones* as well as in a few other rodents and in one weasel. The fleas involved belonged mainly to the genus *Xenopsylla* (*conformis* group) but partly also to that of *Nosopsyllus*. A species of mice (*Mus musculus bactrianus*) was met with but commensal rats appeared to be altogether absent. It is legitimate, therefore, to include Kurdistan among the plague areas where wild rodents form the reservoir of the infection.

In regard to Iraq it was stated by Kaul[69] that plague was absent during the period 1937-44 though in 1938 plague-infected rats were still found in Baghdad. The disease reappeared in February 1945, when 46 cases were reported, mainly from Amara and vicinity (Stowman[147]).

In Turkey a plague epidemic in three villages on the Syrian border took place in March-April 1947. According to Erzin & Payzin, 19 persons were affected, of whom 14 had axillary buboes and 5 septicaemic features; 13 of these patients succumbed. A report[67] on this outbreak added that the infection, apparently present among the rats as well as among man, had been derived from Syria, where plague used to occur in sporadic form among the desert nomads. Actually, after the termination of the outbreak on Turkish territory, 6 cases of plague were notified in the Syrian village of Varta.

(8) *Western Arabia*

Though the early writers dealing with the nosogeography of plague were unanimous in stating that the mountainous area of Assyr, situated in the border regions of present-day Yemen and Saudi Arabia, was an endemic focus of long standing, the last outbreak known to have occurred there in the past took place in 1906. It is, therefore, of great interest to note that the presence of plague in these parts was re-affirmed in 1951 and 1952.

The 1951 outbreak, took place in three villages of the province of Khawlan situated in Yemen near the Saudi Arabian border and lasting from March until September, led to the occurrence of 12 bubonic cases and one instance of pneumonic plague. Anti-epidemic action, including vaccination and dusting with DDT, was taken by a WHO team which had been dispatched after the presence of the disease had been reported at the end of July.

In June 1952 plague was found to be present in the village of Benibashar situated in the Saudi Arabian part of the Assyr area 50 miles from Abha, but as far as is known at present only 4 cases with 3 deaths were observed. Adequate anti-epidemic action was taken, including arrangements to prevent passage of pilgrims from Yemen to Mecca through the affected region.

It would be of great importance to ascertain the species of rodents responsible for these outbreaks. There can be hardly any doubt that wild rodents rather than rats are involved. In fact, it seems probable that the Assyr area forms an outlying part of a vast enzootic area in Western Asia centring in Kurdistan.

(9) *Palestine*

While plague cases or even limited epidemics had been met with in Palestine after the British occupation at the end of the first World War the disease appears to have been absent during the period 1925 40 (Stowman [24]) However, the infection, presumably imported from the Suez Canal Zone reappeared at Haifa in 1941 A considerable epizootic resulted which reduced the incidence of *R rattus* from 63% to 2 7% but only 10 human cases were notified

Jaffa became involved in the winter of 1942-3 when a rat caused outbreak led to 15 cases with 9 deaths Only 1 5% *R rattus* were found at the time and the incidence of *X cheopis* (38%) seemed less than that at Haifa

Plague persisted in both places and occasional instances of the disease were observed also at Tel Aviv The total case incidence was 93 in 1944 and 38 in 1945 when 19 cases with 9 deaths were recorded at Jaffa from October until December In 1946 there were only 13 cases, but the disease became epidemic at Haifa in 1947 when including 3 cases in a secondarily involved village, 17 attacks were notified in June and July (Pollock [115] Haddad & Valero [15])

Europe

(1) *Ajaccio (Corsica) and Taranto (Italy)*

The essential features of the ephemeral outbreaks occurring soon after the second World War at Ajaccio and at Taranto are given in table III

TABLE III DETAILS OF OUTBREAKS OF PLAGUE AT AJACCIO (CORSICA) AND TARANTO (ITALY) IN 1945 [a]

Locality	Dates of occurrence	Origin	Number of cases	Number of deaths	Rats and fleas involved
Ajaccio	*May July* 1945	*Apparently* imported from North Africa	13	10	*R norvegicus* (90%) *R rattus* (10%) *X cheopis*
Taranto	September November 1945	Possibly importation of rags by ship	29	15	*R norvegicus* *R rattus* *X cheopis* *Nosopsyllus fasciatus*

[a] Bernard et al Martorana Schulz[1]

(2) *Malta*

After an absence of over a hundred years plague appeared in Malta in 1917 when a batch of 8 cases with 4 deaths occurred among dockyard

workers and their contacts The first victim was said to have been infected from a sick rat which he found in a box containing stores coming from Mesopotamia where the disease was epidemic (Bernard [11])

A further outbreak ascribed to the importation of straw and hay from North Africa took place from April to November 1936 when 25 bubonic cases with 10 deaths were recorded An epizootic involving mainly *R norvegicus* was found to be responsible for this epidemic *Leptopsylla segnis* was the most frequent rat flea (48 75%) followed by *X cheopis* (37 5%), *Nosopsyllus fasciatus* was rarer (13 75%) (Bernard [11])

Plague again appeared in Malta in June 1945 when an epizootic which involved mainly the area of the commercial port began to manifest itself and led to an epidemic lasting until the end of the year The 75 cases with 20 deaths recorded during this period were followed by 5 further cases with 2 deaths up to June 1946 Infected rodents (mainly *R norvegicus*) were found up to February 1947 (Cauchi [11] Barnett [4])

(3) *Portugal Azores Islands*

Plague outbreaks often showing a high incidence of cases with pneumonic features were recorded since 1908 in several of the Azores Islands (Wu Lien teh [159]) but within recent years only São Miguel Island seems to have been involved An extensive epidemic (744 cases) had occurred there in 1922 3 (Stowman [11]), but since then sporadic cases only averaging 8 per year during the period 1942 8 have been recorded Two cases were notified in 1949 since when plague seems to have been absent

Recent plague outbreaks in the Azores suspected to be due to epizootics among field mice (mulots) were rural in character and appeared chiefly during the seasons when crops were harvested and grain was brought to the granaries [151]

R norvegicus predominated amongst the commensal rodents but black rats (mainly *R rattus*) and *M musculus* were also found *Leptopsylla segnis* was the most numerous flea of these rodents followed by *Nosopsyllus fasciatus X cheopis* being only the third most frequent (Jorge [6])

North and North west Africa

(1) *Egypt*

Fifty five years after Egypt had become free from plague the infection possibly imported from Bombay reappeared in 1899 at Alexandria Port Said the second big port of the country became affected in 1900 Suez in 1904

As soon as the infection was established in the two principal ports it began to travel inland Invading first the chief places of the provinces and

districts but soon spreading to other towns and villages, plague gradually reached most localities of the Nile valley from the coast to Aswan (Wakil,[153] Makar [89]) As shown by statistics compiled by Wakil for the period 1899 1930 (see table IV) the case incidence in Upper Egypt was considerably higher than that in the ports and Lower Egypt

TABLE IV INCIDENCE OF PLAGUE IN EGYPT 1899 1930

Area	Cases		Deaths		Mortality percentage
	Number	%	Number	%	
Ports and Western Frontier Province	3 797	19 6	2 146	20 9	56 5
Lower Egypt	3 201	16 5	1 245	12 1	38 9
Upper Egypt	12 388	63 9	6 881	67 0	55 5
Totals	*19 386*	*100 0*	*10 274*	*100 0*	*52 9*

While plague continued to be comparatively frequent in Upper Egypt where the province of Asyut had become the chief focus the situation in the country in general became increasingly favourable from 1935 onwards as shown by the following figures supplied by Makar [89]

Year	*Total number of cases in Egypt*	*Cases in Asyut Number*	*Cases in Asyut Percentage of total cases*
1931 5	941	389	41 3
1936 7	183	157	85 5

In 1938 only 11 cases were recorded in the country as a whole However while the ports remained free and Lower Egypt had an almost clear bill in 1939 and 1940 there were considerable outbreaks involving 169 and 452 cases respectively in Asyût

As summarized by Stowman [14] from 1941 onwards there was little evidence of plague even in Asyut Unfortunately however as in the first so also during the second World War a serious situation developed in the Suez Canal Zone Outbreaks commenced successively at Suez in November 1943 at Ismailia and in its district in March 1944, and in May of the same year at Port Said where sporadic cases had been noted since 1940 The total incidence of plague from November 1943 to September 1944 was 712 cases (Stowman [14]) The disease reappeared in the Canal ports in February 1945 but the situation gradually improved and by August 1946 the infection had disappeared (Tomich [137])

Alexandria, which had had a clear record since 1935 reported 15 cases with 5 deaths in January 1947 Since then, however plague seems to have been absent from Egypt

The plague seasons in Egypt were, according to Wakil [153] as follows

Zone	Onset	Peak	End
Upper Egypt	March	April	May
Middle Egypt	April	May	June
Nile Delta and Suez	April	June	July
Mediterranean ports	May	July	October

Thus as in other plague affected countries particularly in China the seasonal incidence of the disease in Egypt stood in correlation with the latitude in which the various foci were situated being earliest in the south and latest in the north

The frequency with which the different types of human plague were met with during the period 1904 28 was illustrated by Wakil [153] (see table V)

TABLE V FREQUENCY OF DIFFERENT TYPES OF HUMAN PLAGUE IN EGYPT 1904 28

Zone	Total plague cases	Bubonic and septicaemic cases	Pneumonic cases	
			Number	%
Ports and Western Frontier Province	3 011	425	86	2 8
Lower Egypt	2 660	2 595	6	0 25
Upper Egypt	11 988	10 389	1 599	13 3
Totals	17 659	15 909	1 750	9 9

As will be noted the pneumonic type while rare in the coastal regions and Lower Egypt was comparatively far more frequent in the south of the country

Dealing with the rodents in his classical study on plague in Egypt Wakil [153] enumerated as the species of plague rats most commonly found associated with man *R norvegicus* *R rattus* and *Acomys cahirinus*

The Norway rats were very prevalent in the residential quarters of the port cities *R rattus* being restricted to the dock areas *Acomys cahirinus* was rare in Alexandria and Port Said more frequent in Suez

R norvegicus was scarce in Upper Egypt where according to Petrie & Todd [113] *R rattus* formed 60 6% of the total rat population and *Acomys cahirinus* 38 3%

X cheopis was the most prevalent species of rat fleas throughout Egypt *Leptopsylla segnis* which came next in order was more often encountered in the ports than in Upper Egypt According to data supplied by Wakil [153] *R rattus* was more heavily flea infested than the Norway rats In his

opinion this factor in conjunction with the frequency of the former species of rat was partly responsible for the higher plague incidence in Upper Egypt

(2) *Tripolitania*

As can be gathered from a report received in May 1950 in answer to an inquiry on plague in Africa instituted by the WHO Expert Committee on Plague at its first session, a solitary plague outbreak which was probably *due to importation and led to 12* confirmed and 2 suspected cases, took place in May 1940 in a locality 12 kilometres outside Tripoli on the Tripoli/Garian road

Since no infected rats could be found Modica[101] postulated that this outbreak was due to the importation of plague infected insects or *acari in grain supplies However* no proof for this assumption was obtained

(3) *Tunisia*

Plague, which had been absent from the coastal areas of North Africa west of Egypt since 1822, reappeared in the port of Tunis in 1907 imported, as Jorge[66] believed by ship via Marseilles, France Apart from a pneumonic outbreak which claimed *65* victims in *1929 30* sporadic cases only were observed in Tunis However the disease, appearing usually in the bubonic but occasionally in the pneumonic form was epidemic in the south of *Tunisia in 1920 1* and *fairly frequent during the period 1926 31* when a total of 1 095 cases was recorded, mainly in the districts of Kairwan and Sfax (Stowman[14]) Only 37 cases were notified from 1932 until 1943, the years *1932 4 and 1942 3 being clear However, from August 1944 until March* 1945 an outbreak of bubonic plague (37 cases with 10 deaths) took place at Ferryville (Magrou[88]) and some cases were also noted at Tunis and Bizerta *Tunisia seems to have been free from plague since 1946*

It was claimed that both the 1920 1 and the 1926 epidemics had been due to the immigration of infected wild rodents from Tripolitania However *no definite proof exists that such animals played a role in the* Tunisian outbreaks Gobert[38, 9] claimed to have found suspicious signs in field rats and gerbils but not much reliance can be placed upon his evidence (Wu *Lien teh*[160]) *Ristorcelli*[129] *working in a region where plague was said* to have been present in the past could find no trace of recent epizootics It is noteworthy however that some of the wild rodent species in Tunisia, *particularly the Psammomys* which abound in the south are highly susceptible to experimental infection

Norway rats were by far the most frequent in Tunis but black rats *(R rattus alexandrinus* and *R rattus)* and two species of mice were met with as well The rodents found infected during the period from 1935 to 1940 and again in 1944 belonged to the following species

Species	*Number infected*
R. norvegicus	75
R. r. alexandrinus	29
R. r. rattus	13
M. gentilis	9
M. azoricus	3
Total	129

X. cheopis was the most preponderant species of rat fleas in Tunis but *Leptopsylla segnis* and *Nosopsyllus fasciatus* were also present

(4) *Algeria*

The importation of plague into the Algerian port of Philippeville occurring in 1899 already (Jorge [66]) led at first to no serious consequences the total incidence of the disease in the North African ports up to 1907 amounting to 82 cases

Though plague manifestations became fairly frequent in Algeria from 1911 onwards Grenouilleau [4] felt certain that they were due to repeated importation of the infection by maritime or caravan routes and not to endemicity Up to 1935 major epidemics were observed twice only—in 1921, when 185 cases with 97 deaths were recorded at Aumale and in 1931 when 86 cases of pneumonic plague were notified in the department of Constantine The incidence of the disease from 1935 onwards may thus be summarized

Year	*Total number of cases*	*Remarks*
1935	11	10 of the cases were noted at Philippeville
1936	10	3 of the cases were notified at Algiers
1937	3	All at Algiers
1939	2	
1940	18	
1944	95	94 of the cases occurred at Algiers
1945	11	Small pneumonic outbreak (8 cases) at Oran (Roux & Mercier [128] Gordon & Knies [40])
1946	2	Both at Oran
1950	6	
1935 50	158	Only 2 of the cases were notified from the hinterland

Throughout these years most of the plague cases were observed during the period from August to November

R. norvegicus was the preponderant species at Algiers Bone and Oran but as usually *R. rattus rattus* and *R. rattus alexandrinus* were considerably more frequent in the port area of Algiers than in the city proper (Meunier [93]) To judge from not numerous observations the two subspecies of *R. rattus* were more liable to contract plague than the Norway rats

As reported by Grenouilleau & Carle [43] during the period 1937-44 the rats of Algiers yielded 73 6% of *X. cheopis* as against 19 1% *Leptopsylla segnis* and 6 4% *Nosopsyllus fasciatus*

(5) *Morocco*

Plague in Morocco became first manifest within recent times in 1909 10 when, according to Jorge,[66] a total of 25 cases was observed in military stations of the Casablanca district A violent epidemic, claiming 8,000 10 000 victims took place in 1911 in the district of the Doukkalla and adjacent areas of the hinterland During the period 1912 9 outbreaks of varying severity continued to appear in these parts and the infection spread to the ports of Casablanca and Rabat where, however, not much havoc was caused

Further epidemics occurred from 1922 4 and again during the period 1929 35 but were not on a considerable scale However, the plague situation became quite serious in 1940 from when onwards the case incidence was as follows

Year	*Cases*
1940	1 099
1941	2 337
1942	583
1943	393
1944	227
1945	828

In 1940 the disease appeared first in April among tribes in the southern area of Agadir but spread in September to the Marrakech region In 1942 Casablanca further north became affected, probably through grain transports from the southern foci The case incidence decreased in 1942 to become fairly low in 1943 and 1944 but the infection spread during this period to Rabat, Port Lyautey, and once more to Marrakech In the port of Casablanca, where plague had continued to exist since 1941 a minor outbreak took place in 1944, and a severe epidemic in 1945 The whole of Morocco was free in the following year (Stowman,[11] Kaul[69])

Though plague in Morocco was prevalently bubonic in character, pneumonic cases, sometimes in groups were seen during the 1911 outbreak and a few limited pneumonic epidemics were observed afterwards (Wu Lien teh[159]) The disease did not show a predilection for any particular season of the year (Sanguy[131])

Jorge[66] was inclined to think that, though plague became first rampant in the interior of Morocco it was probably of maritime origin inapparent infection of the commensal rats having become established in the ports and then spreading inland Dealing with the rat population in the ports of Morocco, he came to the following interesting conclusion

Norvegicus is preponderant in the north while in the south it is replaced by *rattus* species especially *alexandrinus* —a murine transition of a mediterranean regime to that of a central African one Even in the north *rattus* tends to spread at the expense of its rival [b]

[b] Le *norvegicus* est préponderant au Nord et cède dans le Sud la place au *rattus* surtout à l alexandrin — c est la transition murine du régime méditerranéen au régime de la Grande Afrique Au Nord même le *rattus* tend à accro tre son domaine aux dépens de son rival "

R. norvegicus were found in the hinterland together with *Meriones* which were sometimes more numerous.

X. cheopis was the preponderant rat flea; *Nosopsyllus fasciatus* and very rarely *X. brasiliensis* and *X. astia* were also found.

It is of great interest to note that, in the opinion of Blanc & Baltazard [12] *human* fleas and lice played an important, if not a preponderant role in the epidemic spread of plague in Morocco. The merits of this contention will be discussed later.

(6) *French West Africa*

Though a few plague cases had been noted in a southern port in 1912 (Jorge [66]), it was in April 1914 that the infection, probably imported by ship from Casablanca, made its real entry into French West Africa in a manner characteristic of the Black Death rather than of modern African outbreaks. As often in the local manifestations of the 14th century, the initial outburst at Dakar was ushered in by a pneumonic phase and it was only 2½ months after the onset that an epizootic became manifest and the bubonic type became prevalent in man. A wide area even including the Cape Verde Islands was reached by the epidemic which, lasting until January 1915, claimed almost 9 000 victims (Wu Lien-teh [159]).

Further plague manifestations in French West Africa became practically restricted to a triangular area in Western Senegal with its tip at Dakar in the west and its northern angle near St. Louis. The incidence of the disease rose after a quiescent period in 1915 and 1916 to reach a maximum of 7 999 cases in 1920 (Damez [1]). The infection was also active from 1928 to 1930, during which period an epidemic at St. Louis described by Lefrou [71] took place, and again in 1934. Then a marked decline lasting until 1942 set in. Numbers of cases of plague from that year onwards are given below (Kaul [69]):

Year	Dakar Circonscription	Senegal	Total
1943	32	266	298
1944	570	69	639
1945	4	54	58
1946-9	0	0	0

It will be noted that in French West Africa, as well as in several of the areas dealt with above, the hitherto favourable plague situation deteriorated considerably during the second World War, when the spread of the infection was facilitated through "a certain amount of war destruction and inadequate storing facilities to meet the war emergency" (Stowman [11]).

The plague season in French West Africa fell into the period from June to August (Sorel [139]). Human plague was in general of the bubonic type but pneumonic cases were met with and were possibly frequent in some outbreaks (Wu Lien-teh [159]).

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As confirmed by recent statistics from Dakar, *R rattus alexandrinus* was by far the most frequent among the commensal rodents *R rattus rattus* less common, and *R norvegicus* not numerous However as pointed out by Cazanove (quoted by Jorge [66]) the comparative frequency of these rodents was apt to vary in different locations the Norway rats of Dakar frequented the port and sewers, *R rattus rattus* was at home in European houses *R rattus alexandrinus* in the habitations of the indigenous population

Plague positive animals found at Dakar within recent years belonged to the following species

Year	*R rattus alexandrinus*	*R rattus rattus*	*R norvegicus*	*M musculus*	*Total*
1935	*10*	*1*	*1*	*2*	*14*
1944	21	33	7	3	64
Totals	31	34	8	5	78

Various wild rodent species have been found plague infected in Senegal and it was suspected that one of them, the giant rat *Cricetomys gambianus*, played a causal role It has to be noted in this connexion that recently all rural foci were situated on the railway line leading parallel to the coast from St Louis to Dakar It was assumed that the storage and transport of ground nuts along this route proved attractive to the wild rodents

Though *X cheopis* was prevalent, in Senegal as in Morocco a possible role of other fleas in the spread of human plague was suspected Attention was paid in this respect not only to *Pulex irritans* but also to *Synosternus pallidus* which though rare on the rats, abounded in human habitations (Kartman [68]) The relative importance of these three flea species in the spread of the infection will be discussed later

Central Africa

(1) *Uganda*

When considering the plague situation in Central Africa it is advisable to deal first with Uganda, where most probably the ancient African centre of the infection mentioned before was situated It serves as a corollary for this assumption that as soon as observations became possible in modern times plague foci of long standing were detected in Uganda (Roberts [1, 3])

Though there was little evidence for the continued existence of the disease in the Protectorate at the close of the last and quite early in the present century, there can be little doubt that the infection was inapparent rather than altogether absent Moreover, it was claimed that during this period the construction of the Uganda Railway, lasting from 1896 to 1901, led to an importation of plague from India Be this as it may it is certain that from 1903 to 1908 most of the ports on Lake Victoria became involved

and that by 1906 the infection had become so firmly entrenched that from then until 1947 it was never absent from Uganda in any year (Hopkins[56])

According to figures culled from the report of Hopkins [6] the mortality reported as due to plague in Uganda during the three decades from 1910 to 1939 was as follows

1910-9	31 305
1920 9	17 410
1930-9	11 387
1910 39	60 102

The situation which as shown above had gradually improved from 1920 onwards became quite favourable during the period from 1940 to 1949

Year	Cases	Deaths
1940	278	268
1941	218	213
1942	354	338
1943	19	0
1944	7	7
1945	4	4
1946	3	3
1947	1	1
1948 9	0	0
1940 9	884	834

Note According to a statement made in the March 1953 issue of the WHO Epidemiological and Vital Statistics Report 14 plague cases with 8 deaths occurred in Uganda early in 1952

As was emphasized by Hopkins [6] the endemic foci in Uganda from which the plague outbreaks originated were invariably situated in rural localities and not in townships

To judge from statistics showing the monthly incidence of the disease from 1935 to 1947 cases occurred throughout the year without showing a regular predilection for any particular season Though human plague was mainly bubonic in character cases with pneumonic features were met with recently by Hennessey [5]

It is interesting to note that in significant contrast to the original foci in Central Asia plague seems not to have become entrenched among the wild rodent species in Uganda

In recent times *R rattus* formed the principal reservoir of the infection in Uganda it was in many of the plague areas practically the only rat found in human habitations and endemicity never became established in regions where this species was absent However *R rattus* did not seem to have invaded Uganda before the beginning of the present century so that it could not have been responsible for the plague outbreaks occurring before that time Its place must then have been taken by *R natalensis* (*Mastomys coucha* auctt) which continues to be the most abundant hut rat

in the parts of the Protectorate not invaded by the black rat *(R rattus)* and which has a flea fauna similar to that of the latter It is likely that *R natalensis* continued to take a limited part in the causation of plague (Hopkins [56])

Xenopsylla brasiliensis was in the opinion of Hopkins, 'the normal initiator of the plague outbreaks in Uganda which, as noted above, invariably started in rural localities *X cheopis*, nearly always confined in the plague areas to large townships helped to carry on the epidemics when such places became invaded but seemed in general not a vector of great importance in Uganda

(2) *Kenya*

The earliest plague outbreak on record in Kenya occurring in 1902 at Nairobi, though not necessarily autochthonous was probably of local origin because, as pointed out by Roberts,[1 3] Mombasa, the only port through which the infection could have entered had been free from the disease for years and remained so until 1912

From 1906 onwards plague began to spread in Kenya but up to the present its incidence has remained usually below the level reached in Uganda (Roberts [1 1]) As summarized by Kaul [69] 600 1,000 cases per year occurred in Kenya from 1926 to 1931, below 300 cases annually from 1932 to 1937, sporadic instances of infection only from 1938 to 1940 However, as shown by the following statistics, the situation became comparatively serious in 1941 and 1942

Year	*Cases*	*Deaths*	*Year*	*Cases*	*Deaths*
1941	781	196	1946	35	11
1942	754	333	1947	55	16
1943	17	13	1948	30	16
1944	18	7	1949	5	2
1945	56	21	1952	59	7

It should be noted that in Kenya as in Uganda the endemic foci of plague were situated in rural areas

A comparatively high incidence of cases with pneumonic features was recorded in some outbreaks recently by Plum,[111] but attention must be paid in this connexion to the following statement (Hunter [60])

> The disease is mostly bubonic in type septicaemic and pneumonic cases are common true inspiration pneumonia is rare

In a study on the transmission of plague in Kenya published in 1950 Roberts [1 4] claimed that *R rattus* was the only rodent involved and that *X cheopis* and *X brasiliensis* served as vectors Human infection was intense in the *cheopis* infested areas probably because this flea which was associated with rats living underground was in far closer and more constant contact with man than *X brasiliensis* which infested the roof rats It is

however important to note that Heisch [1] investigating the Rongai area of Kenya where plague had been endemic for many years was able to prove the presence of the infection not only in *R natalensis* (*Mastomys coucha*) but also in *Arvicanthis abyssinicus* *Otomys angoniensis* and *Rhabdomys pumilio* A pool of 21 fleas (*Dinopsyllus ellobius lypusus*) collected from an *Arvicanthis* burrow also proved positive for *P pestis*

According to Heisch there seemed little doubt that *P pestis* is firmly entrenched in the wild rodents round Rongai and that they are playing an important part in propagating and perpetuating the disease

(3) *Tanganyika*

Since the old trade route from Uganda to the coast led through Tanganyika (formerly German East Africa) it is not surprising to find evidence for the early existence of plague in the latter territory Outbreaks in the Uhehe county of the southern province of Iringa seem to have taken place as early as 1886 and 1889 and the existence of endemic plague in that area was confirmed by the German observers of the 1903-4 outbreak at Iringa (Wu Lien teh [1, 9]) Another endemic focus was detected in 1897 by Koch and Zupitza in Kisiba a locality between the Kagera River and Lake Victoria (Wu Lien teh [1, 9])

Further and sometimes fairly extensive outbreaks continuing in the then German territory from 1906 to 1919 included epidemics in the Gasseni district on the eastern slopes of the Kilimanjaro in 1912 (Lurz [9]) in the Mwanza district and eastern Lake Victoria area in 1913 and at Mwanza township and Dar el Salaam in 1914 After the British occupation manifestations of the disease continued to be frequent in the central and Lake provinces up to 1928 Except in 1931 when 238 cases were recorded plague was inconspicuous or even absent during the period from 1928 to 1936 The incidence of the disease from then onwards was as follows

Year	*Cases*	*Deaths*	*Locality involved*
1937	72	47	Lake province
	61	17	Central province
	2	2	Iringa
	135	66	
1938	3	0	Northern province
1941	2	2	
1948	311	174	Central province
1949	18	14	
1951	263	40	
1952 *	575	98	

* Incomplete

To judge from figures showing the monthly plague incidence from 1936 to 1949 epidemics in Tanganyika usually ran their course during the

period from February to July, reaching their peak in March and April The bubonic type was prevalent but pneumonic manifestations were recorded by the German observers, particularly by Lurz [9]

Black rats, recently identified as *R rattus alexandrinus*, appear to be the only rodents playing a causative role in the Tanganyika outbreaks with *X brasiliensis* as the probable vector

(4) *Belgian Congo*

In compiling this summary on the plague situation in the Belgian Congo advantage was taken not only of the voluminous published literature, but also of an excellently documented report received in July 1950, in answer to the inquiry instituted by the WHO Expert Committee on Plague, from Dr A Duren of the Ministry for the Colonies Brussels

Plague exists in the Belgian Congo in two foci situated in the extreme north east of the colony near the Uganda border—the Lake Albert focus and the Lake Edward focus

Nosogeographically the first of these foci forms part of the plague areas in Uganda because Lake Albert which marks the border between that territory and the affected region in the Belgian Congo, served as a means to convey the infection rather than as a bar to its progress For this reason it seems probable that though the presence of plague in the Lake Albert focus was detected in 1928 only, the disease had been of long standing

Cases in the Lake Edward focus were first notified in 1938 The invasion of this area was evidently due to an importation of the infection from the Lake Albert region, possibly through plague fleas which had been carried by passengers or in goods arriving from there by motor truck (van Riel & Mol [11])

The incidence of the disease in the two foci is shown in table VI

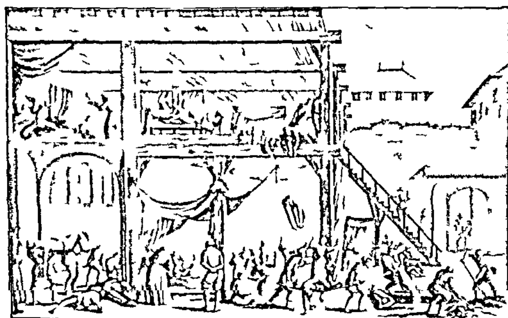
TABLE VI INCIDENCE OF PLAGUE IN LAKE ALBERT AND LAKE EDWARD AREAS BELGIAN CONGO 1928 51

Period	Lake Albert focus		Lake Edward focus	
	Cases	Deaths	Cases	Deaths
1928 37	133	—	—	—
1938 47	249	226	107	93
1948 51	0	66	38	34

It will be noted that thus far the plague morbidity in the Belgian Congo was low The maximal case incidence observed in any year was 65 in

the Lake Albert focus (in 1939) and 29 in the Lake Edward focus (in 1941) The case fatality rate on the other hand was high amounting since 1938 to 91 5% in the former focus and to 87 6% in the latter Commenting upon the severity of the infection the report to WHO stated that instances of ambulatory plague seemed to be altogether absent in the Belgian Congo Cases with lung features were observed and were apparently frequent in the Lake Albert focus in 1939 (van Hoof[5]) when as noted above the incidence of the disease was higher than usual Plague cases seem to be most frequent during the period from April or May to September or October

FIG 2 HOSPITAL FOR PLAGUE PATIENTS IN SPITTELAU VIENNA 1679



Engraving af r L lo co B r ac

Several wild rodent species particularly *Arvicanthus abyssinicus* have been found naturally infected in the Belgian Congo but since these animals had been obtained in the vicinity of villages where rat plague existed they had apparently become secondarily involved

In the Lake Albert focus *Rattus natalensis* (*Mastomys coucha*) was by far the most frequent rodent found in human habitations (98%) Among the few other rodents found *Arvicanthus abyssinicus* was comparatively the most conspicuous It is interesting to note that quite recently an invasion of *R rattus alexandrinus* has been noted in the Kasenyi region but so far no instance of infection has been found in this species *Rattus natalensis ugandae* seems therefore the sole reservoir of plague in the focus

The rodents found in the huts of the Lake Edward focus were *R rattus alexandrinus* (52%) *R natalensis* (32%) and *Arvicanthis abyssinicus* (14%) So far the infection has not been detected in the last species, so that the two first mentioned rodents appear to be responsible for the plague manifestations in the area

Both *X cheopis* and *X brasiliensis* which are considered as the vectors of plague in the Belgian Congo occurred in the Lake Albert focus, the former forming a large majority of the rat fleas in the north and west of the area the latter being almost exclusively present in the east and south In the Lake Edward focus *X brasiliensis* was largely preponderant

Thus far no natural infection has been found in the Belgian Congo in the wild rodent flea *Dinopsyllus ellobius lypusus* (*D lypusus* auctt) but it has been proved to be capable of transmitting plague under experimental conditions *Ctenocephalides felis strongylus*, which in the Belgian Congo infests man in place of *P irritans*, was unable to do so

Madagascar

For a study of the plague situation in Madagascar it was possible to consult, in addition to the voluminous literature, a most valuable summary prepared by the Health Services in Tananarive for the WHO Expert Committee on Plague and containing information up to July 1950

Plague was introduced into Madagascar in November 1898, when a ship bringing a rice cargo from India served as the vehicle of the infection In the following years outbreaks continued to appear in the major ports but never caused much havoc

The infection was again introduced into the port of Tamatave in March 1921, leading to an epizootic and 107 bubonic cases with 71 deaths In June of the same year pneumonic plague appeared at Tananarive on the high plateau of the island and claimed 46 victims in the course of three weeks Four months afterwards foci of bubonic plague became manifest in adjacent localities and signs of rat infection began to be found Since that time the disease has persisted on the high plateau practically all parts of which became successively involved

Occasional outbreaks were noted in the ports as well but these never caused much harm and soon terminated Further, while the outbreaks on the coast continued to be bubonic in nature, the infection showed on the high plateau a marked tendency to lead to secondary lung involvement and thus to produce foci of pneumonic plague Though no major outbreaks of pneumonic plague occurred the incidence of this form of the disease was invariably considerable, averaging according to Le Gall [73] 29% during the period 1935 9 Occasionally the incidence of pneumonic plague was higher, for instance amounting to 56 2% in 1946 (Favarel [?])

The plague incidence in Madagascar from 1933 to 1950 was as follows

Year	Cases	Year	Cases
1933	3 933	1942	181
1934	3 605	1943	234
1935	3 493	1944	184
1936	2 000	1945	185
1937	916	1946	278
1938	630	1947	274
1939	681	1948	240
1940	754	1949	143
1941	272	1950	153

It will be noted that from 1936 onwards the incidence of the disease decreased to reach a level of less than 300 cases per year in 1941 Large scale inoculation campaigns with the live vaccine of Girard and Robic had been started in November 1935 (Robic[1]) and were no doubt responsible for this decrease of the morbidity However as recently pointed out by Lepage the incidence of the disease from 1941 to 1948 though moderate had remained fairly uniform so that apparently a level had been reached which could not be lowered any more through inoculation campaigns alone However application of DDT was started and led to a further decrease in incidence as shown by the following figures

Period	Cases
1st quarter of 1948	135
1949	61
1950	34

Lepage also pointed out that during the early months of 1950 no plague case had been recorded in the Emyrne district where DDT had been used most systematically

Unfortunately further observations did not fully justify this optimistic attitude As noted by Robic in the 1950 and 1951 reports of the Pasteur Institute Tananarive[16, 17] statistics showing the plague incidence in Madagascar not during the calendar but during the epidemic years (1 May to 30 April of the following year) from 1946 onwards were as follows

Epidemic year	Plague cases
1946-47	312
1947-48	278
1948-49	158
1949 50	109
1950 51	241
1951 52	291

In explaining the recently increased plague incidence Robic pointed out that (*a*) since 1949 the disease had been found to be present in forest regions situated beyond the confines of the formerly known endemic areas and that (*b*) in 1951 2 a considerable increase of the plague incidence was noted in the province of Emyrne (131 cases as against 89 in 1950 1)

where pneumonic cases, due evidently to the unrecognized existence o bubonic cases, preponderated Summing up the situation Robic stated tha

> plague is certainly diminishing in the towns and well administered villages everywhe where DDT application can be fully effective e g in and round Tananarive town persists in the rural areas where it would seem it is far from becoming extinct [c]

The incidence of bubonic plague on the Madagascar high plateau wa maximal during the period from 1 December until 31 March and at low eb during June and July Pneumonic cases on the contrary were comparativel more frequent from July to December and less numerous during the ho season from December to February (Le Gall [3])

R rattus alexandrinus and *R rattus frugivorus* are the species o commensal rats commonly met with on the coast as well as on the higl plateau, but the former predominates *R rattus rattus* is preponderant i the forests it occasionally enters store houses but is hardly ever foun inside human habitations

No evidence has been forthcoming thus far that wild rodents play role in the causation of plague, but one species, *Brachytarsomys albicauda* living mainly in the forests, has been found rather susceptible to experi mental infection

There can be no doubt that the black rats, in the first line *R rattu alexandrinus*, form the plague reservoir in Madagascar *X cheopis* is the only flea recognized there as vector of the infection

South Africa

(1) *Union of South Africa*

In compiling this summary advantage has been taken not only of ar ample literature but also of a valuable report on the plague situation in the Union of South Africa from January 1935 to December 1949 prepared by Davis [91] for the WHO Expert Committee on Plague

Plague gained a foothold in the Union of South Africa in 1900 when, on account of the Boer War, large amounts of forage had to be imported from infected South American ports As summarized by Mitchell,[98] during the period from 1900 to 1902 serious epidemics took place at Cape Town, Port Elizabeth, East London, and other centres in Cape Province, as well as at Durban and Pietermaritzburg Further outbreaks occurred in 1903 at King Williams Town, Queenstown and elsewhere, followed in 1904 by a considerable epidemic with an initial pneumonic phase in Johannesburg and its vicinity The infection persisted there as well as in Port Elizabeth and East London until 1905 but seemed to be altogether absent from the Union from 1906 to 1911 In 1912 an outbreak due evidently to a re importation of the infection from eastern ports, occurred

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at Durban As was afterwards realized that epidemic marked the end of the initial plague period in the Union of South Africa which, because it was characterized by the occurrence of rat caused outbreaks in urban areas has been termed the murine phase (Davis[19])

From 1914 onwards outbreaks were noted in remote rural localities first in Cape Province from 1916 onwards also in the Orange Free State whence the infection spread in 1917 to a neighbouring district of Transvaal The total incidence from 1914 to 1918 (when two fatal cases were recorded in the Orange Free State) was 189 cases with 132 deaths Though it was suspected that wild rodents might be responsible for these manifestations of the disease proof for this assumption could be obtained only in 1921 (Mitchell[98])

It was formerly believed that a gradual propagation of plague to the rural areas had taken place the striped mouse (*Rhabdomys pumilio*) contracting the infection in the outskirts of the infected urban centres and passing it on to other wild rodent species (Thornton[14]) Though such a spread took place occasionally (Mitchell[98]) it was of main importance that a direct transport of infected rats and fleas by rail or other means of traffic led to the establishment of three primary distributing centres of wild rodent plague which were situated in south western Transvaal and north western Orange Free State in the Cape midlands and in the Uitenhage district near Port Elizabeth respectively (Fourie[9] Davis[19])

As the result of an expansion of the infection from these primary foci a vast area comprising more than half of South Africa from the Cape to Barotseland in Northern Rhodesia became affected It would seem that a spread beyond the limits thus reached is not to be expected (Davis[19])

The incidence of the disease from 1 July 1919 to the end of June 1949 was as follows

Period	*Cases*	*Remarks*
1919 25	556	Expansion of plague from the original foci due to a continued spread of plague among wild rodents
1925 31	466	Outbreaks mainly in new areas as a result of major extensions of the enzootic area in the karroo (Cape midlands) and northern Orange Free State Spread also into South West Africa and Bechuanaland
1931 7	667	Epidemic in the Orange Free State Extension and consolidation of the infection in other parts of the affected areas
1937-49	646	Continuation of perennial or almost perennial outbreaks in hyperenzootic areas situated in the northern Orange Free State a distri t on the Transkei bord r in the eastern Cape area and in the Uitenhage Port Elizabeth districts Sporadic manifestations in the other parts of the enzootic area (Davis[19]) [d]

[d] [illegible] the [illegible] of h[illegible]man pl[illegible]gue dur[illegible]g the pe[illegible] 195[illegible] [illegible] am[illegible]unt[illegible]g to 14 c[illegible] with [illegible] d[illegible]ath[illegible] [illegible] 1951 [illegible] [illegible] and [illegible]

The incidence of human plague in the Union of South Africa was found to be highest during a season lasting from December to April However, the wild rodent epizootics being unaffected by seasonal changes epidemics have occurred in all months of the year (Davis[21]) Bubonic manifestations preponderated but pneumonic outbreaks have been recorded both during the murine' phase and subsequently in the wild rodent foci recently by Gale[31] and Clark & Goldberg[15]

Though, as stated by Davis,[21] over 100 species and subspecies of rodents and other small animals are at risk of infection and some 60 species of fleas have to be regarded as actual or potential vectors, the main reservoir of wild rodent plague in the Union of South Africa is formed by two gerbils *Tatera brantsi* and *Desmodillus auricularis*, associated with *Xenopsylla philoxera* (*X eridos* auctt, not of Jordan and Rothschild) and *X piriei* respectively Human infection was in most instances not directly derived from the wild rodents or through their fleas the semi domestic *R natalensis* (*Mastomys coucha*) and *R rattus* acting as intermediaries between the primary gerbil reservoirs and man *R natalensis* carried a mixed fauna of wild and commensal rodent fleas while *R rattus* met with in rural areas was infested with *X brasiliensis* the flea considered as the main vector of human plague in the Union

Though as noted above rodents with semi domestic or domestic habits were apt to become involved, instances of a transition of the infection from the wild rodents to urban rat populations have been almost totally absent thus far Only one outbreak at Port Elizabeth in 1938 is on record where a relatively short lived focus was established amongst the commensal rats and gave rise to 28 cases Positive rats were found also at Johannesburg in 1943 1944 and again in 1951, but no general epizootics resulted (Innes[6]) Plague in the Union of South Africa appeared thus within recent times almost invariably in rural localities, where as a rule a few cases only occurred at one and the same time Under these circumstances it is not surprising to find that according to Ferguson,[28] 1 005 individual outbreaks took place from 1920 to 1949 Since he recorded the occurrence of 2 361 cases during this period, the case incidence per outbreak averaged 2 3

(2) *Basutoland*

The recent plague incidence in Basutoland which nosogeographically forms a part of the enzootic areas in the Union of South Africa, was as follows

Year	*Month*	*Cases*	*Deaths*
1935	December	9	9
1936	January February	7	7
1937 41	—	0	0
1942	February	10	4
1943	January March June	27	10
1944	—	0	0

Year	Month	Cases	Deaths
1945	November	8	4
1946-8	—	0	0
1949	November December	92	?
1952		3	3

(3) *Bechuanaland*

Though Ngamiland in the Bechuanaland Protectorate was reached in 1928 by a wave of wild rodent plague spreading from the north west Cape area and the enzootic then established culminated in epizootics in 1934 5 and possibly also in 1939 40 throughout this period only two instances of human plague were recorded—in April 1935 However the reappearance of an epizootic in 1944 led to a major epidemic which lasting from October to December, resulted in 304 cases with 156 deaths Sporadic cases continued up to March 1945 (Davis [18]) The further incidence of plague was as follows

Year	Cases	Deaths
1945	18	16
1946	68	57
1947	2	2
1948	0	0
1949	24	20

As stated in the Bechuanaland Protectorate Medical and Sanitary Report for 1949 [9] signs of an active epizootic were noted during that year in several areas The Gobabis district in South West Africa close to the Bechuanaland frontier was also found to be affected and several fatal cases of human plague were observed there at the end of 1949

Gerbil species (*Tatera* and *Desmodillus*) formed the permanent reservoir of the infection which as will be perceived was apt to flare up every fourth or fifth year *R rattus* and *M musculus* appeared to be absent from Bechuanaland but the multimammate mouse (*R natalensis*) acted as the intermediary between the wild rodents and man

The gerbils were found to be infested with X *eridos* (X *philoxera*) and its close relative *X hipponax* Both these species occurred in addition to X *brasiliensis* on the multimammate mice but the last mentioned flea seemed to be responsible for the majority of human infections (Davis [19])

(4) *Northern Rhodesia*

Suspicious outbreaks were reported in the Luangwa Valley of Northern Rhodesia in 1917 and 1918 *but their plague nature seems not to have been* confirmed (Davis [1]) More recently, however the infection appeared in the Barotse Province which was evidently invaded by wild rodent plague from the south through the Kalahari (Davis [20])

The incidence of the disease in that province since its appearance in 1937 was as follows

Year	Month	Cases	Year	Month	Cases
1937	January	9	1944	February	2
1938 9	–	0	1945 6	–	0
1940	February	4	1947	December	1
1941	–	0	1948	January February	7
1942	October December	14	1949	March	2
1943	December	5	1950	March	2

It will be noted that the plague season fell into the period from October to February when during the midsummer rains day and night temperatures were high (Davis [20]) Occasional pneumonic cases were noted

Though so far no direct proof seems to have been obtained it is most probable that in Northern Rhodesia, as elsewhere in South Africa, gerbils are the primary reservoir of plague, and that the multimammate mouse is instrumental in bringing the infection to man Possibly swamp rats (*Otomys Dasymys Pelomys*) form an accessory reservoir

The gerbils were found to harbour *X philoxera*, *X hipponax*, and occasionally *Dinopsyllus ellobius*, which was the only flea infesting the swamp rats It is interesting that *X philoxera* a notorious vector was with one exception found solely in those areas of Barotseland where plague was enzootic However since *X hipponax*, which abounded in the regions free from *philoxera* might be a vector, no undue stress ought to be laid upon the absence of the latter flea

R natalensis was infested by the wild rodent fleas mentioned above and by *X brasiliensis* which no doubt was of main importance in conveying the infection to man (Davis [0])

North America

(1) *United States of America*

The incidence of human plague in the USA from 1900 to 1950 may be summarized as follows

(*a*) *Rat caused epidemics* (Mohr [10])

Locality	Yea	Cases	Deaths	Remarks
San Francisco Calif	1900 4	120	114	Plague existed probably before 1900 (see below)
	1907 8	186	92	
Seattle, Wash	1907	3	3	2 of these patients and probably also 3 suspected victims had pneumonic plague Rat infection persisted until 1917 (Fricks [30])

Locality	Year	Cases	Deaths	Remarks
New Orleans La	1914 5	31	10	
	1919 21	25	11	
Pensacola Fla	1920	10	4	
Galveston Tex	1920	18	12	
Beaumont Tex	1920	14	6	
Los Angeles Calif	1924	41	34	33 of the patients had lung features but these were in part secondary in nature (Wu Lien teh[1, 9])
Totals		448	286	

(*b*) *Infections contracted from wild rodents or through their fleas* (after Link[6])

State	Year of detection of Wild rodent plague	Human plague	Cases	Deaths
California	1908	1908	80	52
Oregon	1935	1934	1	1
Utah	1936	1936	1	0
Nevada	1936	1937	1	0
Idaho	1936	1940	1	1
New Mexico	1938	1949	6	3
Arizona	1938	1950	1	0
Totals	1908 51		91	57

Remarks While most of these cases occurred singly one pneumonic outbreak of wild rodent origin took place at Oakland California in 1919 and claimed 13 victims

Taken at their face value these statistics suggest that in the USA as well as in South Africa and some countries of South America an initial murine phase of plague eventually led to wild rodent infection However according to some observers the appearance of the disease in North America was due not to its recent introduction by the sea route but to an early importation through wild rodents which coming overland across the Bering Strait from Central Asia brought *Pasteurella pestis* with them as a population regulator (Jacobsen[61] Meyer[96]) Meyer[96] pointed out in this connexion that it was difficult to believe in as rapid a spread of wild rodent plague as ought to have taken place had the infection been recently introduced Likewise he maintained that as a rule wild rodent plague in North America

" remained confined to foci and no convincing evidence has been produced that some wide ranging animal has started new epizootics "

While it would be out of place to discuss this point at the present juncture it should be noted here that it is difficult to believe in the fascinating hypo thesis set forth above for the following reasons First should plague have come overland from Central Asia it should have appeared in Canada earlier than in the USA Actually however it appeared in that Dominion much later than in the USA and was no doubt imported from there Further as will be discussed in chapter 2 the plague strains isolated in the USA

are biochemically different from those in Central Asia Finally, i
no means certain that the usually given date of entry of the ir
into the USA was the actual one Kinyoun[71] felt sure that plag
been present in San Francisco in 1898 already and quoted a report c
that "in all probability, the disease had existed on the Pacific Coa
1896 Seeing that plague had reached the coast of China as e
1867, one might even wonder if it had not been carried to Californ
before 1896, so that there was far more time for a spread of the ir
to the hinterland than is usually allowed for

As will be gathered from the first of the statistics inserted abo
caused plague, though repeatedly appearing in the USA, has never
marked tendencies to spread or persist This is all the more rem
because, with the exception of Washington State the commens
in the plague affected localities showed a considerable infestatio
X *cheopis* *R norvegicus* was the most preponderant rat species

Wild rodent plague was present not only in the States where it
human infection, but also in others as shown by the following (Li

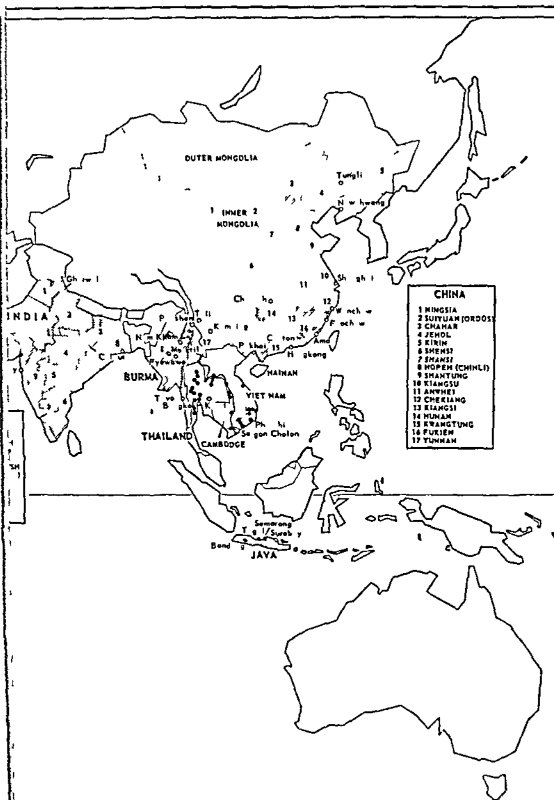
State	*Year of detection*	*State*	*Year of detect on*
California	*1908* *	Arizona	*1938*
Montana	1935	New Mexico	*1938*
Oregon	1935	Colorado	1941
Idaho	*1936*	North Dakota	*1941*
Nevada	*1936*	Oklahoma	*1944*
Utah	1936	Kansas	1945
Wyoming	*1936*	Texas	*1946*
Washington	1937		

* Wild rodent plague had evidently existed in the Contra Costa county of C
since 1903 at least

The low incidence of human infections derived directly from th
rodents or through their fleas is in striking contrast to the larg
comprising 131 counties in 15 States, where evidence of plague
these animals has been found Discussing this problem, Meyer[90]
that the disease

> occurs among wild rodents in wooded or rural districts uninhabited or only
> inhabited by man but human contact with the infective agent probably is establ
> exceptional instances

Eskey & Haas[25] in their classical study on 'Plague in the W
part of the United States , came to the conclusion that at least
groups of rodents constituted the great primary reservoirs of the in
—the ground squirrels which were widely involved in the coastal r
and in the northern part of the Intermountain Plateau , the woo
which formed the plague reservoir in the southern deserts and
the prairie dogs, which harboured the infection in the plateau reg
Arizona and New Mexico Mohr[10] endorsed this opinion but also in



The dots and hatching represent areas w
human cases were reported from 1948 to

While as noted above the danger of a spread of plague to man through direct contact with wild rodents or through their fleas is slight, secondary involvement of the rats or other rodent species living near man might greatly enhance the chances for human infection

The possibility of such a transition had been claimed by the advocates of an overland introduction of the disease from Central Asia who pointed out that up to 1908 large numbers of ground squirrels had been imported into San Francisco for culinary purposes (Meyer[94]) It was also suspected that an infection recently derived from wild rodents had been responsible for the epizootic causing the 1924 Los Angeles epidemic (Wu Lien teh[1 9]) and that the presence of plague among the rats of Tacoma (Washington State) in 1942 and 1943 had been due to an importation of the infection from the hinterland through grain transports (Hundley & Nasi[59]) Wild rodent fleas had been found upon several occasions on commensal rats and isolated instances of rat plague had been detected in urban centres as well as in rural areas of California (Meyer & Holdenried[97] Mohr[10])

Working recently on a Californian ranch round which wild rodent plague was present Meyer & Holdenried[97] were able to prove the presence of the infection in rats (*R rattus rattus* and *R norvegicus*) which were in part infested with ground squirrel fleas *L segnis* was the preponderant specific parasite on these rats while *Nosopsyllus fasciatus* was rare and *X cheopis* absent However in the opinion of the authors the wild rodent fleas might have been able to maintain the infection which they had introduced Meyer & Holdenried came to the important conclusion that

" on numerous occasions rural plague has swept with destructive force through native rodent populations with little if any danger to human beings but when domestic rats (*Rattus*) or mice (*Peromyscus Microtus* or *Mus*) which may live in man s immediate environment and the fleas of which will attack and thus infect him are involved a much more serious problem arises

Fortunately this potential danger is fully realized due attention being paid to rodent and flea control in and round human settlements

(2) *United States of America — Hawaii*

Plague became manifest in Honolulu in November 1899 a few months after patients suffering from the disease had been found on two ships arriving from Hong Kong The infection not only persisted for about 10 years in Honolulu but spread to adjacent districts as well as to other islands of the archipelago

Locality	*Period*	*Cases*
Oahu Island		
Honolulu	1899 1910	187
Other places	1902 7	41
Kauai	1901 6	15
Maui	1900	9
	1930-2	6
	1938	1

Locality	*Period*	*Cases*
Hawaii Island		
Hilo sector	1900 12	43
North Hilo	1918	2
Hamakua district	1910-45	111
	1949	1
	Total cases 1899 1949 =	416

As will be noted within recent years human plague was present in the Hamakua district of Hawaii Island only, where after a clear interval a case was recorded in 1949 and where rodent infection persists to date Positive results were also obtained in 1949 with two batches of pooled tissues from Maui rats

The district of Hamakua contained many sugar plantations and, as stated by Eskey,[4] human plague infection was contracted in the cane fields rather than in the houses *R rattus alexandrinus* formed half of the rat population in the district but *R norvegicus* and *R hawaiensis*, a small mouse like animal rarely entering houses were also met with *X cheopis* constituted 70% of the rat fleas

The mortality from human plague was high since as stated by Hampton,[46] *360 of the 397 patients seen from 1899 1933 and all 17 observed* from then up to 1944 died[e]

(3) Canada

An importation of plague from the USA being anticipated, a large scale wild rodent survey was made in Western Canada in 1938 No positive results were obtained (Gibbons[31])

However, in 1939 the prevalence of an epizootic among the ground squirrels of Alberta was noted, and bacteriological findings proving the presence of plague were made in one of these animals taken at Stanmore a locality about 180 miles (290 km) north of the boundary separating Canada from Montana USA Since, as noted above wild rodent plague had been found to exist in that State since 1935 there can be no doubt that the infection had spread from there into Canada

Further evidence of plague in the Alberta ground squirrels was found in 1939 42 and again in 1945 Spread of the infection from Alberta to an adjacent region of Saskatchewan was noted in 1946 (Humphreys & Campbell[58]) Pools of ground squirrel fleas were found positive for plague in 1947 both there and in Alberta

The ground squirrels involved in Canada belonged to the species *Citellus richardsoni richardsoni* They harboured *Opisocrostis labis O tuber*

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[e] Besides in the Hawaii archipelago plague has been reported within recent years in another Pacific island New Caledonia where early in 1941 a village near the seaport of Goro situated about 30 miles east *of Noumea the capital* became affected Up to March 1 nine cases with six deaths were recorded eight of which were stated to have occurred among pupils of a native school Prior to this epidemic which was bacteriologically confirmed an unusual mortality among the "brush or coconut tree rats in the Goro region had been noted It was also stated that plague was latently endemic in small areas of the colony

The entire population of the plague affected area was vaccinated Plague serum and sulfathiazole were used for treatment

culatus and *Oropsylla rupestris*, three species of fleas found able by Eskey & Haas [5] to convey plague under experimental conditions.

It is reassuring that according to Humphreys & Campbell [9] commensal rats seemed not to have colonized to any extent in Alberta. However, they infested the large municipalities of Saskatchewan.

Thus far the existence of human plague has not been proven in Canada, but it is possible that one fatal case occurred in Alberta before the existence of wild rodent infection had been established. The man in question was breeding minks which he fed with ground squirrels from a locality later found to be plague-affected. Several of the minks died and their breeder got scratched whilst skinning one of these animals (Gibbons & Humphreys [3]).

South America

(1) *Venezuela*

Plague, believed to have been imported from Trinidad, appeared in 1908 in the port of La Guaira and soon spread from there to Caracas, the capital of Venezuela, where it continued to occur until 1919, leading to a total of 204 cases with 99 deaths.

Even before this "urban" phase had come to an end, the disease had spread in 1910 to rural areas of Miranda State, in one district of which it continues to exist. An adjacent region of Aragua State, reached by the infection in 1939, also remained involved. As stated by Isaac Riaz [63], the two affected districts cover an area of 1 000 km^2 (386 square miles), 1% of Venezuelan territory.

The plague incidence in the two affected regions from 1910 to 1951 was:

Miranda State

Year	*Cases*	*Year*	*Cases*
1910	35	1928	10
1911	18	1932	10
1914	16	1933	7
1919	110	1950	5
		1951	7

Total cases 1910-51 = 218

Aragua State

Year	*Cases*	*Deaths*
1939	11	8
1943	17	5
1945	7	3
1949	2	1
1939-49	37	17

At Caracas *R norvegicus* formed 98% of the rat population and *X cheopis* 95% of the rat fleas as against 3 4% *X brasiliensis* (Hecht [48])

The commensal species (*R norvegicus, R rattus rattus* and *rattus alexandrinus*) were also present in and around the settlements of the plague affected rural areas and were no doubt responsible for the appearance of plague in man, *X cheopis* and *X brasiliensis* serving as vectors However, Isaac Riaz [63] postulated with much reason the existence of wild rodent foci where the infection was carried over during the often prolonged periods of its absence from human settlements Actually, instances of natural plague were found in two wild rodent species, *Heteromys anomalus* and *Sigmodon hirsutus* Isaac Riaz claimed in this connexion that the fleas of the genus *Polygenis* which formed the bulk of the fauna on the wild rodents, were particularly apt to produce smouldering enzootics because they did not become blocked when ingesting plague bacilli It is, however of interest that sometimes a high percentage of *Xenopsyllae*, particularly *X brasiliensis*, was found on the wild rodents (Hecht [49] Isaac Riaz [63]) An increased incidence of the latter flea was noted in June 1943 one month before the appearance of the outbreak in Aragua State The presence of 52 2% *X brasiliensis* and 20 3% *X cheopis* in May 1942 did not lead to an epidemic, but one might venture to suggest that a high prevalence of *Xenopsyllae* on the wild rodents was of importance only when at the same time plague was active amongst these animals The necessity for a coincidence of these two factors might explain why in the rural areas of Venezuela years with manifestations of human plague are separated by long periods of quiescence

(2) *Brazil*

It is generally accepted that plague, imported by the sea route first appeared in Brazil in 1899 when Santos and a few months afterwards São Paulo lying inland from that port, became infected From then onwards up to 1906 Rio de Janeiro Fortaleza in the State of Ceara Pernambuco Rio Grande do Sul, and other ports became successively involved From 1907 onwards the infection began to spread to inland cities and towns but disappeared since 1934 rapidly from these centres while persisting in rural areas which remain in part involved to date (Barreto & Castro [8]) The total incidence of the disease from 1899 to 30 June 1949 is shown by the following approximate figures (Moll & O Leary [103] A Castro communication to WHO Expert Committee on Plague 1950)

Period	*Cases*
1899 1929	5 638
1930-4	535
1899 1934	6 173

Period	Cases	Deaths
1935-9	1 223	490
1940-4	812	205
1945-9	1 040	182
1935-49	3 075	877

Total cases 1899-1949 = 9 218

Details of the plague incidence in Brazil from 1935 to 1949 are given in table VII (A Castro communication to WHO Expert Committee on Plague 1950)

TABLE VII NUMBER OF CASES OF AND DEATHS FROM PLAGUE IN BRAZIL 1935-49

Year	States of								Total	
	São Paulo		Rio de Janeiro		Minas Geraes		North East Brazil			
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
1935	2	1	—	—	—	—	569	232	571	233
1936	31	21	—	—	—	—	318	115	349	136
1937	1	0	—	—	—	—	35	15	36	15
1938	—	—	12	8	—	—	134	53	146	61
1939	4	2	—	—	—	—	107	43	111	45
1940	—	—	—	—	—	—	255	53	255	53
1941	—	—	7	4	—	—	295	83	302	87
1942	—	—	—	—	3	0	32	7	35	[illegible]
1943	—	—	—	—	—	—	66	22	66	22
1944	—	—	—	—	—	—	134	36	134	36
1945	—	—	—	—	—	—	192	42	192	42
1946	—	—	—	—	34	23	298	48	332	71
1947	—	—	—	—	7	3	81	8	88	11
1948	—	—	—	—	—	—	356	54	356	54
1949[a]	—	—	—	—	—	—	41	4	41	4
1935-49	38	24	19	12	44	26	2 973	815	3 074[b]	877

[a] Until 30 June [b] One recovering case in Sergipe State in 1946 not included

The plague incidence in Brazil was 55 cases with 10 deaths in 1950 while in 1951 there were 20 cases with 4 deaths all in the north east of the country

It will be noted that except for one outbreak in Minas Geraes State in 1946-7 which has been dealt with by Macchiavello & Martins de Almeida [87] from 1943 onwards north eastern Brazil alone was involved The situation there is given in table VIII

As shown by table VIII, the situation was serious in four of the six States concerned, particularly in Pernambuco

TABLE VIII CASES OF AND DEATHS FROM PLAGUE IN THE STATES OF NORTH EASTERN BRAZIL 1935 49

Period	Alagoas		Bahia		Ceará		Parahyba		Pernambuco		Piauhy		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
1935 9	74	23	79	31	317	95	14	6	673	297	16	6	1 173	4[illegible]8
1940 4	256	36	126	39	108	21	—	—	312	105	—	—	80[illegible]	201
1945 9[a]	53	8	319	48	249	44	23	3	354	53	—	—	998	1[illegible]6
1935 49	383	67	524	118	674	160	37	9	1 339	455	16	6	2 973	815

[a] Until 30 June

As was to be expected the seasonal incidence of plague in Sao Paulo in the south of Brazil was different from that farther north Most of the cases of bubonic plague in São Paulo occurred during the sultry days of summer with the peak in January (Oliveira,[107] Moll & O Leary [103]) In marked contrast to this a pneumonic plague outbreak *at São Paulo* in 1936 took place during the winter in July (de Moura Albuquerque [101]) and the same held true of an earlier pneumonic plague epidemic observed at Santa Maria in the State of Rio Grande do Sul in 1912 (Wu Lien teh [159])

The period of maximal plague incidence in Rio de Janeiro was from September to January, commencing thus earlier than in São Paulo (Rangel quoted by Moll & O Leary [103])

As shown by monthly figures supplied to the WHO Expert Committee on Plague by Castro *for the period from 1935 to 1949* the seasonal plague incidence in the north east of Brazil was not as clear cut as in the south of the country

Month	*Number of cases*
January	233
February	194
March	235
April	190
May	103
June	75
July	403
August	312
September	372
October	364
November	250
December	242

Still the incidence of plague in north eastern Brazil during these years was comparatively highest from July to October thus roughly corresponding to the seasonal period mentioned by Barreto for Pernambuco He claimed an earlier onset for Ceara which is situated farther north (May June) and stated that in both States the period of maximal plague incidence coincided with the harvest season

While as noted above occasional pneumonic epidemics occurred bubonic plague was largely preponderant 95 7% of the cases observed in Brazil from 1936 to 1945 being of this type as against 2 16% pneumonic and 1 7% septicaemic cases (Barreto & Castro [8]) The plague mortality was generally low and as will be discussed in a subsequent chapter of this monograph atypically mild cases were frequent

Though as will be recorded later natural plague has been found in a whole series of Brazilian wild rodents it was held by Macchiavello [8] and other observers that these animals did not form a primary reservoir of the infection but were secondarily or even accidentally involved

While *R rattus* was preponderant in the rural areas of the north east Norway rats were most common in some of the ports and in São Paulo State In Rio de Janeiro this species and *M musculus* were more frequent than *R rattus* and *rattus alexandrinus* Though *X brasiliensis* occurred everywhere side by side with *X cheopis* the comparative frequency with which the former flea was present on the rats increased pari passu with the latitude of the regions in question (Barreto & Castro [8]) *X cheopis* was considered as the plague vector in Brazil (Macchiavello [9])

(3) *Argentina*

Apparently by passing Buenos Aires and the Argentinian ports on the Paraná River plague became manifest first in South America at Asuncion situated far inland in Paraguay which had been reached in April 1899 by an infected steamer From this original focus the disease was soon carried back to Rosario and other river ports in Argentina at the end of the year also to Buenos Aires

The initial period of the infection thus established was followed first by a stage during which plague was carried inland by rail then by further progress of the pest to remote regions in the interior where wild rodent foci were created A wide area extending from the provinces of Jujuy and Salta on the Bolivian border in the north to Patagonia in the south thus became gradually involved (Sussini [113])

As pointed out by Barrera [6] generally speaking the inland foci of plague in Argentina fell into two groups

(*a*) Those in the central part of the country particularly in the provinces of Rio Negro La Pampa Mendoza and San Juan with a sparse population and no agriculture Since grain stores which might have attracted the

rodents to the settlements were absent contact with infected animals was restricted to chance meetings in the fields, and the incidence of human plague was consequently low

(*b*) Those in the north especially in Santiago del Estero, Tucuman and Salta where accumulation of agricultural products attracted the rodents to the settlements and houses, and the incidence of human cases was accordingly higher

The number of plague cases in Argentina from 1899 to 1930 amounted to about 6,200 (Moll & O Leary[103]) The recent incidence of the disease was as follows

Year	*Cases*	*Deaths*	*Year*	*Cases*	*Deaths*
1931	37	24	1941	56	28
1932	61	19	1942	38	19
1933	63	25	1943	2	1
1934	45	40	1944	107	63
1935	15	10	1945	?	3
1936	31	21	1946	10	3
1937	20	18	1947	4	0
1938	15	8	1948	12	4
1939	5	4	1949	0	0
1940	228	192	1951	1	0

Note In 1940 the provinces of Santiago del Estero Cordoba and Tucuman were mainly involved in 1944 Salta and Jujuy Provinces

Though these figures show the trend of the infection rather than the exact incidence of the disease, which was probably higher, they indicate that on the whole the plague situation in Argentina was favourable within recent years The ports were free after 1931 with the exception of a small outbreak (8 cases with 3 deaths) in November 1946 at Buenos Aires (Moll & O Leary[103]) Considering the large extent of the areas involved the morbidity in the rural districts was as rule a rather low and moreover as is characteristic of the manifestations caused by wild rodents, human cases were usually not grouped together but appeared in numerous foci which were independent of one another (Outes,[108] Villafañe Lastra et al[15]) It must be noted, however that pneumonic cases were comparatively frequent and that repeated though usually limited pneumonic plague epidemics were observed Miyara et al[100] recorded 27 such outbreaks with a total of 222 deaths and one instance of recovery for the period 1913 38

Villafañe Lastra et al[15] noted the interesting fact that within recent years there was a notable increase of outbreaks taking place in autumn and winter whereas formerly the plague incidence had been highest in summer

Among the commensal rodents of Argentina, Norway rats were predominant in the towns as well as in the rural areas *R rattus rattus* was less common *R rattus alexandrinus* least frequent except in the coastal provinces *X cheopis* was the prevalent flea species on these animals (Moll & O Leary [103])

As summarized by Macchiavello,[86] natural plague was found in the following wild rodent species of Argentina

Cavia pamparum	*Hesperomys murillus cordovensis*
Galea musteloides (2 subspecies)	*Lepus europaeus*
Cavicella (Microcavia) australis (2 subspecies)	*Sylvilagus brasiliensis gibsoni*
Graomys griseoflavus (2 subspecies)[f]	

Two of the fleas found on these rodents *Delostichus* (formerly *Parapsyllus*) *talis* (on *Cavicella australis*) and *Polygenis* (formerly *Rhopalopsyllus*) *platensis cisandinus* (on *Gr griseoflavus*) have been found capable of transmitting plague by their bites and attacking man (Barrera [5])

The relative importance of the commensal rats and of the wild rodents as well as the question of a transition of plague from the former to the latter or vice versa have been the subjects of much debate No doubt can exist that originally the infection spread from the commensal rats (probably from *R norvegicus*) to the wild rodent species (Villafañe Lastra et al [illegible]) and it is also certain that foci have become established where wild rodents alone are responsible for the causation of human plague as well as for the perpetuation of the infection However in other localities a spread of the disease from the wild to the commensal rats was observed and the latter then played a subsidiary or even a preponderant role in the causation of human attacks Barrera [6] maintained that opportunities for contact between the wild and the commensal rodents were present particularly in the agricultural areas of North Argentina where both were attracted by the grain stores and a study of the available literature confirmed that involvement of the commensal rats in the chain of infection was far more frequently noted there than in the central provinces

(4) *Bolivia*

Though the presence of plague in southern Bolivia was bacteriologically confirmed in 1928 only there can be little doubt that outbreaks due primarily to an importation of the infection from North Argentina have occurred since 1921 The somewhat incomplete information on these manifestations of the disease culled from Moll & O Leary s compilation as well as from other sources (Veintemillas [151] Mealla [9] Prado Barrientos [114] Siles [137] Cors [16] Benavides [10]) may thus be summarized

[f] Some of the other species of wild rodents occasionally found naturally infected in Argentina are [illegible] in Annex I (page 6[illegible])

Following its importation by the sea route into the port of Callao in 1903 the disease spread along the coast of Peru, most of the principal ports becoming infected within two years and eventually invaded 10 of Peru s 20 departments as well as the three special provinces of Tumbes Callao and Moquegua The situation became worst in the coastal departments of Lambayeque Libertad and Lima as well as inland in Cajamarca (Eskey [3] Moll & O Leary [103]) The former three areas remained infected to date while Cajamarca reported cases in 1948

The total incidence of plague in Peru up to 1952 may be summarized thus

Period	*Cases*	*Annual average*
1903 12	8 865	886
1913 22	6 922	692
1923 32	4 642	464
1933–42	1 087	109
1943 52	738	74
1903 52	22 254	445

Note The maximal yearly incidence was in 1908 (1 691 cases) A second peak (1 200 cases) was reached in 1926

As shown by the classical study of Eskey [23] the central part of Peru situated between the 7th and 13th degrees of latitude and specially the areas between the 7° and 9° with an average annual mean temperature between 69°F (20 5°C) and 71°F (21 7°C) suffered most from plague There the infection spread rapidly to rural as well as urban communities produced more cases than elsewhere in the country and showed little tendency to disappear spontaneously However the degree of rat infestation of the houses was also of importance Thus the ports of Paita and Mollendo though situated well away from the zone where the climate favoured the spread of plague suffered heavily because their wooden buildings were attractive to the rats Lima on the contrary though open to inroads of the infection as far as the climatic conditions were concerned had a lesser morbidity than these two ports because of having better-class houses

Generally speaking the annual plague epidemics in Peru tended to reach their peak during the summer months However there as elsewhere the plague seasons fell into an earlier period in areas where the winter months were warm than in localities with a colder climate (Eskey [3])

Human plague in Peru was mostly bubonic Pneumonic plague was rare in general but one outbreak claiming 21 victims in the department of Junin was mentioned by Moll & O Leary [103]

As stated by Eskey [3] *R norvegicus R rattus* and *R rattus alexandrinus* were common in the towns of northern Peru while in the central and southern coastal areas as a rule Norway rats greatly exceeded the other two species In the rural districts near Lima *R norvegicus* and *R rattus alexandrinus* were found *X cheopis* was the most common rat flea and according to Eskey the only important plague vector

Locality	Period	Cases	Remarks
Southern Zone			
Loja Province	1921 40	1 411	Plague which has probably been present since about 1919 continues to occur to date

Having become exclusively rural in character the infection persists in Ecuador, mainly in the mountainous areas of Chimborazo and Loja Provinces as shown by the following data

Year	Cases	Deaths	Year	Cases	Deaths
1941	39	14	1947	21	—
1942	5	2	1948	40	38
1943	15	9	1949	19	5
1944	36	11	1950	28	2
1945	38	16	1951	35	19
1946	45	19	1952	38 *	

Information incomplete

In general plague in Ecuador was most rampant in December of low incidence in June However in Loja Province outbreaks occurred chiefly in the dry season from May to December (Moll & O Leary [103])

While generally bubonic cases were most common the incidence of pneumonic plague was comparatively high in the mountainous areas Saenz Vera [1 9] noted in this connexion in 1941 that while only 43 out of the 8 000 odd cases observed in the coastal areas since 1908 were pneumonic there were 194 instances of pneumonic plague (22 2 %) among the 874 cases recorded in Chimborazo Province from 1913 onwards invariably in rural localities According to Murdock [105] three pneumonic epidemics took place in Ecuador during 1939

Period	Province	Locality	Deaths
January February	Chimborazo	Riobamba	17
April		Columbe	14
September	Loja	Cofradia Loja	At least 7

Except in the case of pneumonic plague the plague mortality in Ecuador was as a rule low Two peculiar forms of the disease angina pestosa (tonsillar plague) and viruela pestosa (plague pox) were met with there

While *R norvegicus* was preponderant in Guayaquil *R rattus alexandrinus* and *rattus* were more frequent than the first mentioned rat in the sierra towns which had become infested only after the construction of the railway (Martinez [90]) As maintained by Macchiavello [83] commensal rats (*R rattus rattus* and *rattus alexandrinus*) were also prevalent in the rural areas of the interior He considered that these rodents were of prime importance in the causation of the plague outbreaks there the guinea pigs amply bred in the houses for culinary purposes playing merely an auxiliary role While in the mountains as well as elsewhere in Ecuador *X cheopis*

was the common vector of the infection, Macchiavello came to the interesting conclusion that at altitudes over 9,100 feet (2,770 m), where conditions were unfavourable for that flea, *Nosopsyllus londiniensis* took an important part Since its vector capacity was not high, human cases tended to be sporadic

Apart from the above mentioned focus comprising adjacent parts of Ecuador and Peru, wild rodents played no role in the causation of plague in the former country

* * *

As will be gathered from the account rendered above in many of the countries dealt with the incidence of plague has markedly decreased within recent years and in some the disease has ceased to be manifest for the present However, even apart from the fact that some areas remain seriously involved it is not yet possible to be complacent about the plague situation in the world

As its history teaches plague has often shown a decline even if left alone but was apt to flare up again in due course Hence, while appreciating the strenuous efforts now often made to combat the scourge, one should be careful not to ascribe to the measures implemented what might really be the outcome of a periodicity of the infection

While human plague has become a quite preventable as well as an almost always curable disease it is disappointing to see that on account of administrative or financial difficulties it is still often impossible to take ful advantage of the modern methods of treatment and prevention

Moreover, even universal success in these directions will not eradicate the infection which will continue to lurk among the rodents unless effective action against these pests can be combined with the therapeutic and pro phylactic work

There is hope that, in addition to the campaigns undertaken for the specific purpose of plague control general progress in health and wealth will gradually prove inimical to the commensal rats It is to be feared however, that the vast primary reservoirs of the infection among the wild rodents will remain unassailable for a long time to come

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Chapter 2

THE PLAGUE BACILLUS

CLASSIFICATION

Following a proposal made by Rahn[141] in 1937 the plague bacillus *Pasteurella pestis* together with *P pseudotuberculosis* *P multocida* *P haemolytica* and *P tularensis* was classed in the family of Parvobacteriaceae and in the genus *Pasteurella* Trevisan in the 5th and 6th editions of Bergey s *Manual of determinative bacteriology*

As van Loghem[106] pointed out recently with much reason this classification is rather unfortunate because it places micro organisms such as the plague and pseudotuberculosis bacilli which possess normal dimensions among the Parvobacteriaceae characterized by their exceptionally small size Van Loghem proposed to class these two species in a new genus called *Yersinia* in honour of the discoverer of the plague bacillus

No doubt this recommendation deserves serious consideration because it was always doubtful whether or not the plague and pseudotuberculosis bacilli which while closely related to each other stand apart from the pasteurellae in general should be grouped among them In view of the fact however that so far the change in nomenclature advocated by van Loghem seems not to have attracted much attention it did not appear advisable to adopt it for the purposes of this monograph

MORPHOLOGICAL CHARACTERISTICS

Normal and involution forms

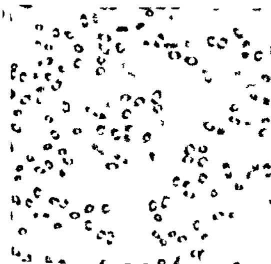
P pestis which belongs to the class of Gram negative micro organisms appears typically as a short ovoid bacillus showing bipolar staining but is actually characterized by great variability in shape (see fig 5 9) Variations in length are frequent so that both short coccoid forms and longer rods (Kokkentypus and Stäbchentypus of the German




6




8



PASTEURELLA PESTIS

FIG 5 LYMPH NODE FLUID IN HUMAN BUBONIC PLAGUE (× 1 500)

FIG 6 LUNG SMEAR IN HUMAN PNEUMONIC PLAGUE (× 1 500)

FIG 7 24 HOUR CULTURE IN BROTH OF VIRULENT STRAIN (195/P) (× 1 500)

FIG 8 EARLY STAGES OF INVOLUTION FORMS ON HORMONE AGAR (× 1 500)

FIG 9 INVOLUTION FORMS IN LYMPH NODE OF SULFADIAZINE TREATED MOUSE (× 1 500)

treatment showed signs of involution, while smears from the control animals displayed a normal morphology of the bacilli Roux & Mercier[118] stated that the sputum smears of pneumonic plague patients treated with sulfathiaziole showed alteration of the structure of the micro organisms and similarly Pollitzer (unpublished observation) could see in the sputum of such a patient recovering under sulfadiazine treatment a gradual replacement of typical plague bacilli by involution forms

An interesting observation, suggesting that atypical bacillary forms may be prevalent at the initial stage of plague manifestations was made by Magrou & Brisou[113] in the course of an epizootic of rat origin among guinea pigs kept in the laboratory

At first no characteristic gross lesions could be detected in the carcasses of these animals and smears from their organs showed only small, round Gram negative corpuscles which seemed in part vacuolated Cultures made at that stage of the epizootic remained sterile or growth was tardy Later on typical forms were found in the smears and normal growth was obtained Magrou & Brisou were afterwards able to detect similar atypical forms in smears from the bubo punctate of patients who had been inoculated or suffered from pestis minor

Haffkine (quoted by Dieudonne & Otto[47]) had pointed out that growth on dry agar promoted the appearance of involution forms This matter was further studied by Zammit & Alcock[00] who found that in drier media not infrequently coccoid and very short bacillary forms (generally associated with some oval bipolar staining forms) were present while in smears from moist agar especially from the condensation water, more typical forms were seen When investigating this problem in a more general manner, Epstein[48] came to the conclusion that bipolar staining was not peculiar to the *Pasteurella* group but could be demonstrated to some degree in other kinds of bacteria as well if they were cultivated in media rich in water

These observations seemed to suggest that phenomena of osmotic pressure have an important bearing upon the morphology and staining properties of *P pestis* (Petrie[1 9]) Interesting observations in this direction were made by Dudchenko[45] when studying a plague like capsulated micro-organism This bacillus formed under unfavourable conditions a firm capsule, and intracellular pressure consequently became high Dudchenko assumed that the latter factor was responsible on the one hand for the displacement of the endoplasm to the poles of the bacterial cell and on the other hand for the formation of various misshapen involution forms However as will be noted below, some recent workers have tried to explain the presence of bipolar staining in another manner

Capsule and envelope

Though Kitasato[9] and many other observers were of the opinion that the plague bacillus was a capsulated organism Rowland[119] came to the conclusion that a capsule was present under certain conditions only—at the site of inoculation in experimentally infected rats and in bacilli which

FIG 10 FIRST DESCRIPTION OF THE PLAGUE BACILLUS BY YERSIN

Les morts avant d'être enterrés
au cimetière sont déposés pendant
une heure ou deux dans une sorte
de cave. Ils sont déjà dans leurs cer-
cueils et recouverts de chaux. On ouvre
un de ces cercueils, j'enlève un peu la
chaux pour découvrir la région crura-
le. Le bubon est bien net, je l'enlève
en moins d'une minute et je monte
à mon laboratoire. Je fais rapide-
ment une préparation et la mets
sous le microscope. Au premier coup
d'œil je reconnais une véritable purée
de microbes, tous semblables. Ce sont de
très petits bâtonnets, trapus, à extré-
mités arrondies et assez mal colorés
(Bleu de Loeffler)

had been grown in media containing serum Rowland *claimed on the* other hand that the plague bacilli possessed a slimy envelope ' which was not easily visible in unstained preparations but could be demonstrated in indian ink preparations and differed from the capsule in having no definite outer limit

The presence of such an envelope was again postulated by Schutze[160] in 1939, by Bhatnagar[20] in 1940 and recently by Seal[16] in 1951 However Sokhey,[17] who published in 1940 the results of a systematic study of this matter came to the conclusion that while the plague bacillus possessed a capsule under all circumstances the envelope seemed to be only an unstained capsule plus a halo which is produced by a peculiar settling down of the fine particles of India ink at a distance from the capsule due to the operation of physical forces Amies[3] while categorically refuting this explanation confirmed through studies of wet Indian ink preparations with the aid of dark field examination that the so called envelope of *P pestis* is nothing more than a particularly well developed bacterial capsule

The presence of a capsule (membrane cellulaire) was also confirmed by P an Tchan & Pochon[156, 157] when studying the cytology of the plague bacillus in preparations coloured according to Robinow s method with crystal violet The cellular membrane was seen to disintegrate and finally to disappear under the influence of a specific bacteriophage

It is of great interest to note that, generally speaking a capsule though *typical for P pestis, is not present under all circumstances* Chertnik[9] stated in this connexion that the organism was provided with a capsule as long as only sufficiently moist media were used for cultivation Most favourable conditions for the development of capsules were created when the plague bacilli were cultivated at a temperature of 36° 37°C in an atmo sphere containing 25% 30% carbon dioxide—preferably on Martin s peptone agar Chertnik also claimed that addition of rabbit blood greatly favoured capsule formation, but it is noteworthy that according to Amies[3] this was apt to favour the production of non capsulated variants

Confirming the findings of earlier workers, Chertnik[79] maintained that the pseudotuberculosis bacillus showed no definite (ausgesprochene) capsule and was consequently devoid of the membrane antigen ' possessed by the plague bacillus—observations supported by the postulations of Schutze,[160] Bhatnagar[20] and Seal[16] that the pseudotuberculosis bacilli had no envelope

Amies,[3] who worked with the avirulent Tjiwidej strain used by Otten as a plague prophylactic, was able to produce uncapsulated (naked) forms uncovered by any extracellular substance either through repeated subcultivation or by treating the growths with aqueous solvents such as potassium thiocyanate depriving in both ways the organisms of their main immunogenic antigen He noted that the change to the non capsulated type produced through subcultivation was heralded by the appearance of

large ovoid cells with a strong affinity for fuchsin which were first in minority but rapidly became predominant on further subculture

Motility

Though a few observers have claimed that the plague bacillus shows motility the overwhelming majority of experts are agreed that it is an immotile organism Since it has been proved on the other hand that the pseudotuberculosis bacillus is typically motile, it would seem at first glance that this difference would greatly facilitate the otherwise rather difficult differentiation between these two species Unfortunately however considerable practical difficulties are encountered in this direction On the one hand plague strains may show such marked Brownian movement that great experience may be necessary to decide whether a given bacillus is immotile or not On the other hand the pseudotuberculosis bacillus although it is usually motile when grown at temperatures below 26° C does not as a rule show this property when incubated at 37° C (Kossel & Overbeck [101] Arkwright [4]) Moreover since it has been demonstrated that the motility of this micro organism is due to the presence of flagella one must expect that immotile O forms occur (Schutze [159])

Favarissova [1] who tested the motility of 17 pseudotuberculosis strains cultivated at 14° 16°C with the aid of hanging drop preparations or of Levinthal s method found that cultures kept for a long time in the laboratory were apt to be immotile but that motility could be restored by frequent subculture at suitably low temperatures especially if bouillon containing serum or semi solid agar were used for this purpose Favarissova came to the conclusion that motility tests were of value for the differentiation of plague and pseudotuberculosis bacilli

As stated by Seal [16] the difficulties confronting microscopical examinations for motility may be obviated by the use of stab cultures in semi solid agar at an incubation temperature of 25°C or a little less He claimed that if tested in this manner pseudotuberculosis strains showed a characteristic tree like growth as an evidence of motility whereas the development of *P pestis* remained restricted to the immediate vicinity of the stab It must be noted however that prolonged growth of this organism in stab cultures is apt to lead to the formation of tuft like outgrowths (Pollitzer [13])

Granules and nuclei

As summarized by Pollitzer [13] in 1936 the presence of granules or nucleus like formations in *P pestis* had been noted by a few workers

Schultz [157] observed granules in plague cultures kept for four years without subcultivation and considered them as a means of providing for the survival of the organisms Subcultures showed typical growth and the granules did not stand heating at 50° C for 1½ hours so that they were certainly not in the nature of spores

Fusco & Patane [58] [59] stated that granules may be demonstrated in smears from plague cultures or even sometimes in those directly taken from a plague bubo if an ammoniacal

solution of methylene blue be used for staining Results were best if cultivation was made on beef serum solidified with 15% glucose Similar granules were found in certain allied organisms, including the pseudotuberculosis bacillus if these were treated in an identical manner

The existence of nucleus like formations in the plague bacillus has been claimed by some workers such as Pokrovskaya[133, 134] Lugovaya & Lebedeva[10] and Tshernobaev[181] who all stated they had confirmed the nature of these bodies with the reaction of Feulgen and Rossenbeck It should be added that more recently Korobkova[99] reported the occurrence of giant forms with a well differentiated nucleus in the transparent smooth colonies produced in plague cultures through bacteriophage action

Studying the cell division of *P pestis* with the aid of Robinow s method of acid hydrolysis and Giemsa staining, Wei, Tchan & Pochon[191] noted the presence of a chromatic corpuscle near each pole of the resting bacterial cells In the course of division these two corpuscles first became joined together in the form of a dumb bell and then separated into four granules As a result, each daughter cell contained two chromatic corpuscles which again became situated near the poles of the micro organisms Wei and his co workers came to the conclusion that the bipolar appearance of the plague and allied bacilli was due not to protoplasmic condensations near the poles as had been assumed by most previous workers, but to the presence of the chromatic corpuscles described above They admitted however, that reserve materials serving for the nutrition of the bacterial cells might be accumulated round these bodies

As shown by further investigations by P an Tchan & Pochon,[1 6 1 7] the action of a specific bacteriophage interfered with the normal cytological evolution of the plague bacillus the chromatic granules at first becoming swollen and fused together then transformed into filaments and finally disintegrating Using tannin as a mordant and Robinow s method of coloration with crystal violet the same workers showed that bacteriophage action besides preventing further cell divisions, led not only to a gradual disintegration and final disappearance of the cell membrane but also to the appearance of granules coloured with crystal violet P'an and his co workers assumed that these bodies were abnormal constituents of the bacterial cell their appearance being accelerated by action of the bacteriophage

It should be noted that Shabaiev & Pletnikova[16] had previously described the presence of metachromatic granules in plague bacilli grown on peptone agar which had been sterilized at 125° 130° C or on media containing phosphate compounds

Modes of reproduction

The modes of reproduction of *P pestis* were recently studied by Scanga[15a] with the aid of the electronic microscope He found that this organism which is characterized by a marked pleomorphism also showed reproduction modes different from that of simple binary fission in the transverse diameter, such as atypical fission or multiplication through the formation of small coccoid bodies which appeared either at the pole of

the bacterial cells or laterally Scanga postulated therefore that *P pestis* was capable of multiplying through a process of gemmation

CULTURAL CHARACTERISTICS

Growth limits and requirements

It is gratifying to note that the rather incomplete knowledge formerly available in regard to the environmental conditions and substrates suitable for the growth of the plague bacillus has been supplemented through recent systematic studies

Working on the basis of previous observations by Sokhey[1 0] Sokhey & Habbu[1 1] determined the optimum and limiting temperatures for the growth of the plague bacillus in broth Cultivation was possible within a temperature range of from $-2°C$ to $+45°C$ with an optimum at 27° 28°C when the yield was about five times larger than that at 37° C It should be noted in this connexion that as previously established by Sokhey,[171] a temperature of 37 5° C was optimal for the growth of sparsely seeded plague bacilli on blood agar

Sokhey & Habbu[174] further established that at an incubation temperature of 28° C plague bacilli developed in broth within the pH range 5 0 9 6 Growth was fairly satisfactory when the pH of the medium was between 6 6 and 8 0 and was maximal within the range 7 2 7 6

Studying the number of viable plague organisms in broth cultures Sokhey[1 1] reached the important conclusion that the growth resulting after an incubation of 48 hours bore no relation to the total quantity of the medium or to its surface area but was directly proportional to the circumference of the latter[a] In order to obtain identical results in successive examinations it was therefore essential to use invariably tubes of the same internal diameter and to keep them during incubation invariably in the same position and in an undisturbed state free from shocks

Interesting results were obtained by Sokhey & Habbu[175] when studying the rate of growth of the plague bacillus in broth Two stationary phases were noted which lasted from 0 to 1 hour, and from 21 to 24 hours respectively After each of these phases, there was a logarithmic growth phase

This phenomenon seemed to be due to the fact that at first growth took place in the body of the medium only where the oxygen tension was 0 72 ml 0 75 ml per 100 ml of the medium The initial growth phase came to an end in about 21 hours when the oxygen content had fallen to 0 04 ml per 100 ml However a second growth phase on the surface of the medium commenced about three hours afterwards

In a further study Sokhey[1 3] recorded the interesting observation that a lag phase was absent when *P pestis* were implanted into the filtrate of

Sokhey[1] stated in a later paper (195) We have since found that although this observation is not strictly correct it still does hold because the growth in the body of the medium which we had failed to note is so very small as compared with the growth at the circumference

a broth culture previously used to grow the same organisms for about six hours—obviously because during this period of preliminary growth the organism through lysis had added to the medium some essential nutrient material which permitted of fresh growth in geometric progression from the very start Sokhey concluded, therefore,

> that the lag phase was caused by the lack of some essential nutritive materials in the culture medium and that this lack was made good by the lysis of the organism itself The lag phase represented the period during which the lysis was going on and continued until a sufficient amount of the nutrient had been added to permit of growth in geometric progression

Studying the nutritional requirements of the plague bacillus, Rao [14] found three amino acids—proline, phenylalanine and cystine—to be essential for its growth, while glycine, though not indispensable, exerted an important stimulating action As established by Rao and confirmed by all subsequent observers accessory growth factors (' bacterial vitamins) were not required for the cultivation of the plague bacillus

As shown by further investigations of Rao,[143] besides the above mentioned amino acids serine alanine, glutamic acid, tyrosine and methionine were of service as nitrogen and energy sources for the growth of the plague bacillus and were therefore suitable for enrichment of the media Glucose and lactic acid were found to be the best and at the same time the least expensive carbon sources that could be used to supplement the media

Rao [14] found two chemically defined protein free media—hydrolysed gelatin + cystine and proline + phenylalanine + cystine + glycine—suitable for cultivating plague bacilli but pointed out that in general hydrolysates *of simple proteins (such as gelatin or casein) in which* the presence of essential amino acids is ensured will prove to be the most useful

In fact as recently described by Sokhey, Habbu & Bharucha [176] casein hydrolysate proved an excellent substrate in the preparation of plague vaccine

In order to study the influence of haematin and certain other compounds upon the growth of the plague bacillus Rao [143] used a basic medium containing 12 amino acids He found haematin highly active in speeding up growth while cozymase thiamine and *nicotinic acid exerted* a similar but less marked influence The combination of haematin thiamine and nicotinic acid had the greatest effect On the basis of these investigations Rao suggested that

> the four growth stimulants may be essential components of the [bacterial] cell being synthesized in the course of growth and that their occurrence and ready availability in the environment will greatly facilitate the rapidity of growth and invasion of the organism "

This postulation is supported by the above *mentioned observations* of Sokhey

Results similar to those of Rao were recorded by Yaoi et al[196] who studied the nutritional requirements of the plague bacillus with the aid of Mueller & Johnson s hydrolysed casein medium to which cystine had been added

Further studies on the growth requirements of *P pestis* supplementing the work of Rao may be summarized as follows

Doudoroff[43] succeeded in cultivating plague strains in an ammonia salts glucose medium to which cystine and phenyl alanine had been added Berkman[8] was able to grow plague and pseudotuberculosis bacilli in a medium containing 15 amino acids inorganic salts and glucose Haemorrhagic septicaemia pasteurellae (pasteurellae sensu stricto) on the other hand failed to develop in this medium or in hydrolysed gelatin unless nicotinamide and pantothenic acid had been added

A chemically defined fluid medium which duplicated the amino acid composition of casein was recommended for the cultivation of *P pestis* by Rockenmacher et al[147] Tested with 17 virulent and 10 avirulent strains this medium probably because it met the requirement for an amino acid balance proved fully satisfactory even when small inocula were used However it was necessary to add haematin or blood or to resort to inoculation under reduced atmospheric pressure to obtain success with an identically prepared agar medium

Studying the nutrition of three virulent and three avirulent strains on a basal medium containing glucose ammonium sulfate and other inorganic salts Hills & Spurr[87] established the interesting fact that the growth requirements of *P pestis* varied in accordance with the temperature of incubation At temperatures of 32°C or below a medium containing phenylalanine valine isoleucine cysteine methionine and haemin proved optimal The medium best suited for growth at 36°C had to contain in addition alanine leucine serine threonine biotin and pantothenate A mixture of twenty amino acids was required when biotin and pantothenate were omitted

The most likely explanation of this discrepancy seemed to be that at higher temperatures katabolic processes leading to the death of the organism are accelerated relative to the synthetic processes and the organism becomes dependent on a wider range of preformed nutrients

Englesberg[47] working with one avirulent plague strain found that this required as nitrogen and sulfur sources for growth DL phenylalanine DL valine DL isoleucine DL methionine and thiosulfate In the presence of DL methionine thiosulfate was found to be replaceable only by either L-cysteine sulfide or sulfite and in the presence of thiosulfate DL methionine was replaceable only by DL homocysteine or L-cystathionine *P pestis* had therefore a dual sulfur requirement which seemed due to its inability to synthesize cysteine and methionine from inorganic sulfate

Though the practical aspects of plague laboratory work, including methods of cultivation, will be dealt with later in chapter 5 it is necessary to discuss here the general features of the growth of *P pestis* on or in plain media agar gelatin and broth

(*a*) *Growth on agar* The youngest forms of plague colonies becoming visible on agar plates about the end of the first day of growth were described by Markl[114] as being extremely delicate translucent, colourless, and coarsely granulated with strongly scalloped edges After 48 hours, two types of colonies could be distinguished—type I colonies which were roundish, sharply defined, and raised with steep edges, grey white in reflected light and bluish in transmitted light , and type II colonies which were larger, with a central nucleus and a more or less broad delicate peripheral zone with wavy edges , the central part of these type II colonies was sometimes poorly developed, so that they appeared uniformly flat and delicate (Albrecht & Ghon[2] quoted by Petrie[1 9])

Markl[114] maintained that these two types originated from the early colonies described above either directly or through intermediate stages and were due to an adaptation of the bacilli to adverse conditions Thus on plates to which plague toxin had previously been added the two types were conspicuous whereas on control dishes colonies of the early type grew, which only gradually changed over

From these statements though the descriptions given do not tally in all details, it appears likely that the two above mentioned colony types of the plague bacillus correspond to the smooth and rough variants described by recent workers Colonies of these variants are shown in fig 11 and 12

FIG 11 NORMAL COLONY OF P PESTIS ON AGAR (S TYPE)

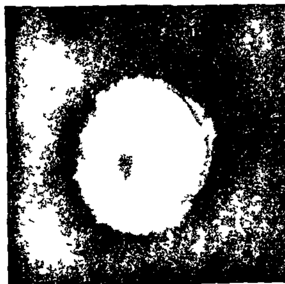
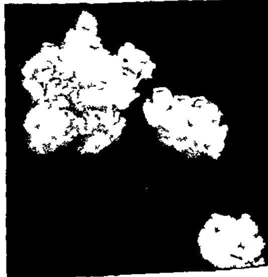


FIG 12 ATYPICAL P PESTIS COLONY (R TYPE) INDUCED BY PHAGE ACTION



As first noted by Yersin [197, 198] in 1894 especially in cultures directly implanted with material from the animal or human body, different rates of growth may be conspicuous leading to considerable differences in the size of the colonies sometimes to the development of veritable giant colonies (usually of type II) beside minute dwarf colonies These differences were not however of a stable character, so that by subcultivation of either type again large and small colonies were obtained (Frosch [56])

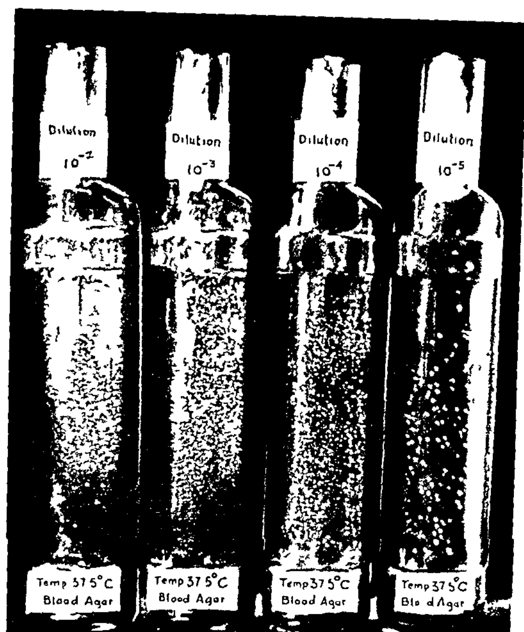
Cultivating under suitable conditions on agar slants one obtains a delicate greyish white layer of growth which when touched with a platinum needle displays a slimy consistency considered by MacConkey [111] to be characteristic for the plague bacillus in contrast to the pseudotuberculosis bacillus

(*b*) *Growth on gelatin* After incubation at 20° 22° C for 2 3 days plague colonies develop on gelatin dishes in the form of fine semi transparent dots with a protruding coarsely granulated centre and a delicate transparent peripheral zone with wavy edges Impression films from these colonies show a characteristic microscopic appearance owing to the presence of loops and filaments Kossel & Overbeck [101] who first noted this feature ascribed differential diagnostic importance to it but this claim was not confirmed by other observers who found loop formation sometimes absent in plague colonies and present in those of the pseudotuberculosis bacillus It should also be noted that on gelatin as on agar further development of the plague bacillus leads to marked variations in the appearance of the colonies A further drawback is that in the case of contaminated material liquefaction of the gelatin by some of the concomitant micro organisms may interfere with continued observation of the cultures Nevertheless some workers reported success with contaminated material when incubating the gelatin dishes at low temperatures Zlatogorov [91] even recommended freezing of the inoculated plates for a period of 2 3 days

(*c*) *Growth in nutrient broth* According to the descriptions usually given growth of the plague bacillus in broth starts with the appearance of floccules on the bottom and at the side of the culture tubes or flasks followed by the formation of a surface film from which threadlike strands hang down As shown by Haffkine [80, 81] this growth from the surface may be facilitated by the addition to the medium of a little oil or other fatty fluid which forms a superficial film and thus facilitates the formation of the hanging strands or stalactites towards which stalagmites grow up from the bottom of the tube or flask One may however obtain such growth without any addition if one carefully avoids vibration of the cultures

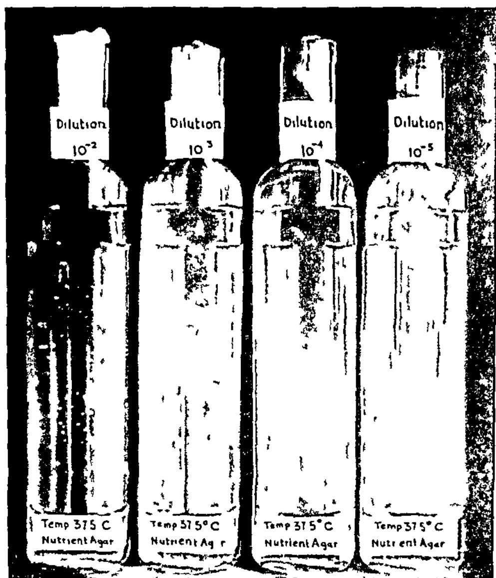
The question of the extent to which this peculiar growth in broth is characteristic for the plague bacillus has been the subject of much debate Some observers ascribed differential diagnostic importance to this feature maintaining that the plague bacillus invariably produces such sedimentary

FIG 13 P PESTIS COLONIES ON BLOOD AGAR



growth in bouillon whereas the pseudotuberculosis bacillus renders this medium uniformly turbid However as first shown by Schutze,[158] this holds true for the smooth form of the pseudotuberculosis bacillus only, whereas its rough form produces a flocculent growth in bouillon More important still, the recent observations of Wats & Puduval [189] recorded below have proved that, similarly, the smooth variant of the plague bacillus produces uniform turbidity in bouillon Since however, the persistence of the plague bacillus in a purely smooth form seems to be an exception

FIG 14 P I ESTIS COLONIES ON NUTRIENT AGAR



rather than the rule it is not surprising to find that usually this micro organism produces a sedimentary type of growth in nutrient bouillon

(d) Growth from small inocula Though as discussed above plain media may be used for the cultivation of the plague bacillus it is as a rule impossible to initiate growth on ordinary agar or in nutrient broth unless large inocula are implanted The following observations proving this curious point were made by Pollitzer[13] in *Plague A manual for medical and public health workers*

Drennan & Teague[44] found that when agar plates were sown sparsely with plague bacilli no growth occurred They ascr bed this phenomenon to the presence of inhibitory

substances formed during preparation of the media and found that additi
sulfite neutralized this supposed effect Noting that growth of plague ba
place in the vicinity of a variety of micro organisms developing on ag
assumed that these bacteria exerted a similar neutralizing action

The above observations were confirmed by Meyer & Batchelder[113] and a
et al [2 2] who found that different sarcinae stimulated the growth of
plated out from diluted suspensions

Schutze & Hassanein[161] could induce growth from small inocula not or
sodium sulfite to the media but also by adding blood or sterilized broth
variety of bacteria or by keeping the plates under anaerobic conditions
the difficulty of initiating growth of plague bacilli lying widely scattered o
was due to an oxygen sensitivity of the micro organisms the two autho
that sodium sulfite and blood acted as reducing agents The action of st
cultures was in their opinion due to the presence of substances of an enz

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a growth inhibition similar to that of the plague bacillus pseudotuberc
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Wright[193] believed that plague bacilli failed to grow from small inoc
because the constituents of this medium had become oxidized To overco
culty he recommended adding the peptone to the broth at an early stage of
He confirmed that plague bacilli were readily destroyed by exposure in thin
agar surface to the action of air particularly at 37° C This could be
(a) reducing the oxygen content of the atmosphere to some point betwee
1 5/ and (b) adding to the media blood (0 1%) serum (10%) or sodium s
or even less)

Observations regarding this subject made since 1936 may be s
thus

Schutze,[160] working with virulent and avirulent rough ar
plague strains, found practically all these variants equally unable
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Sokhey[171] recorded experiments confirming the observatio
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but stated it as his opinion that a possible reduction of the oxyg
by the addition of these reducing agents was not the sole expl
the increased growth He considered blood agar as the medium
for the cultivation of sparsely seeded plague bacilli on solid
fig 13 and 14) and as has been noted already, found an incubati
ature of 37 5°C optimal for this purpose

Rao[143] discussing the findings of Schutze & Hassanein sugg
the growth stimulating factor present in sterile culture filtrates wa
while the factor preventing growth in the presence of molecular o

Further investigations in this direction were recently recorded by Herbert[86] This worker was unable to cultivate three virulent and two avirulent plague strains with the aid of the synthetic media successfully used by Rao and by Doudoroff Anaerobically his strains grew in a mixture of 20 amino acids and glucose but to obtain aerobic growth from small inocula it was necessary to add whole blood or a peptic digest of blood to the media Herbert found that the factor in peptonized blood responsible for this effect was haematin but that growth from small inocula was also obtainable if pure recrystallized haemin was added to plain agar

While admitting that intrinsic differences between the strains used by him and by Rao and Doudoroff respectively might have played a role Herbert laid stress upon the fact that large inocula had been used by Rao and initially also by Doudoroff

Herbert like Sokhey,[171] doubted that the failure of initiating cultivation with small inocula was mainly a problem of oxygen tension as had been assumed by some previous observers Sharing the opinion of Rao[113] he postulated that the growth inhibition was due to a production of hydrogen peroxide by aerobically growing plague bacilli and ascribed the effect of blood and blood substances to their utilization by the organisms for the synthesis of catalase which decomposes hydrogen peroxide

The fact that the media could also be made suitable for the growth of small inocula through the addition of substances such as sodium sulfite thiolacetate or animal charcoal could be explained as well by this hypo thesis For as stated by Herbert the effects of these reducing substances could be ascribed either to a direct chemical reaction with the hydrogen peroxide or to their reaction with oxygen in the medium which would prevent the formation of H O Animal charcoal on the other hand catalytically decomposes hydrogen peroxide

The following procedures were recently recommended to promote growth from small inocula

Samsonov[153] reported success when organ lysates prepared from the spleen or liver of laboratory animals were added to the media Bistrenin et al [1] used pepsin trypsin digests of albuminous substances for the same purpose while Jastchouk[88] obtained favourable results with Fieldes peptic blood broth It should be kept in mind however that as recently emphasized by Girard[67] ordinary blood agar is as suitable as these more elaborate media for obtaining satisfactory cultures of the plague bacillus regardless of the size of the inoculum

As noted above one of the methods recommended by Schutze & Hassanein[161] for promoting the growth from small inocula was cultivation under anaerobic conditions Good results with this procedure were recently reported by Girard & Neel [6] Using an agar medium which had been pre

substances formed during preparation of the media and found that addition of sodium sulfite neutralized this supposed effect Noting that growth of plague bacilli also took place in the vicinity of a variety of micro organisms developing on agar plates the assumed that these bacteria exerted a similar neutralizing action

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Schutze & Hassanein[161] could induce growth from small inocula not only by adding sodium sulfite to the media but also by adding blood or sterilized broth cultures of a variety of bacteria or by keeping the plates under anaerobic conditions Believing that the difficulty of initiating growth of plague bacilli lying widely scattered on agar plates was due to an oxygen sensitivity of the micro organisms the two authors suggested that sodium sulfite and blood acted as reducing agents The action of sterilized broth cultures was in their opinion due to the presence of substances of an enzymic nature

Whereas in the experience of Schutze & Hassanein certain other pasteurellae showed a growth inhibition similar to that of the plague bacillus pseudotuberculosis bacilli grew well even if small inocula were used

Wright[195] believed that plague bacilli failed to grow from mall inocula in broth because the constituents of this medium had become oxidized To overcome this difficulty he recommended adding the peptone to the broth at an early stage of preparation He confirmed that plague bacilli were readily destroyed by exposure in thin layers on an agar surface to the action of air particularly at 37° C This could be prevented by (a) reducing the oxygen content of the atmosphere to some point between 0 5% and 1 5% and (b) adding to the media blood (0 1%) serum (10%) or sodium sulfite (0 05% or even less)

Observations regarding this subject made since 1936 may be summarized thus

Schutze[160] working with virulent and avirulent, rough and smooth plague strains, found practically all these variants equally unable to develop when thinly seeded on plain agar only one smooth avirulent strain from Java grew well under these circumstances The plague bacilli thus behaved differently from other pasteurellae such as *P aviseptica* and *lepiseptica*, the rough variants of which developed well on plain agar even if small inocula were implanted

Sokhey[171] recorded experiments confirming the observation that the addition of defibrinated blood, serum copper sulfate, or sodium sulfite promoted the growth of sparsely seeded plague bacilli on agar surfaces, but stated it as his opinion that a possible reduction of the oxygen tension by the addition of these reducing agents was not the sole explanation of the increased growth He considered blood agar as the medium of choice for the cultivation of sparsely seeded plague bacilli on solid media (see fig 13 and 14) and as has been noted already found an incubation temperature of 37 5°C optimal for this purpose

Rao[143] discussing the findings of Schutze & Hassanein suggested that the growth stimulating factor present in sterile culture filtrates was catalase, while the factor preventing growth in the presence of molecular oxygen was probably hydrogen peroxide

Further investigations in this direction were recently recorded by Herbert[86] This worker was unable to cultivate three virulent and two avirulent plague strains with the aid of the synthetic media successfully used by Rao and by Doudoroff Anaerobically his strains grew in a mixture of 20 amino acids and glucose but to obtain aerobic growth from small inocula it was necessary to add whole blood or a peptic digest of blood to the media Herbert found that the factor in peptonized blood responsible for this effect was haematin but that growth from small inocula was also obtainable if pure recrystallized haemin was added to plain agar

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pared with a special brand of peptone (Uclaf No 17) and to which glucose had been added for stab cultures, these two authors were able to start growth from small inocula in instances where surface cultivation had given negative or more tardy results Repeated subcultivation under anaerobic conditions seemed to exert an unfavourable influence upon the virulence of the strains

An elaborate method of promoting the growth of plague bacilli devised by Devignat[3] was to bubble air through broth cultures in a special apparatus An abundant yield was obtained quickly and uniform suspensions suitable for agglutination tests were obtained It should be noted in this connexion that the plague bacilli growing in aerated broth cultures did not become linked together in chains, as is the case in ordinary bouillon cultures, but remained isolated

Devignat & Schoetter[30] noted that repeated passage of plague bacilli through aerated media was apt to lead to the appearance of involution forms and resulted in a loss of virulence of the strains However, Devignat[33] was able to establish that their virulence remained unimpaired if quicklime instead of potassium hydroxide was used for the removal of water and carbon dioxide from the air to be passed through the culture tubes He was of the opinion that radioactivity conferred upon the air by the potassium hydroxide had been responsible for the loss of virulence

Although he failed to initiate growth when implanting single plague bacilli directly upon nutrient media, Tchan[1 8] was able to cultivate such organisms isolated with the aid of a micromanipulator, when using a preliminary enrichment method described by him in the following terms

> We then deposited a few droplets containing the single cell on a cover slip in a hanging drop under oil immersion After a few hours during which a number of cell divisions took place we injected a little peptone water by means of a micro pipette into the droplet From ten separate single cells we thus obtained three cultures in the oil immersion [b]

Tchan pointed out that the proportion of positive results obtained with the aid of this method was in accord with the observations of Otten[1?5] who found in cultures of his avirulent plague strains about one third viable and two thirds dead micro organisms

It is curious to add that Yu[199] obtained quick and abundant growths of plague bacilli when using media prepared with rat meat or rat liver and heart infusions He claimed that the time required for cultivation of the micro organisms on these substrates was about half that necessary in the case of beef extract media and that the yield obtained with the new method was considerably higher

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b Nous avons alors déposé sur la lamelle à l intérieur de la chambre à huile des gout elettes contenant la cellule isolée Apres quelques heures au cours desquelles se produisent plusieurs divisions cellulaires nous injectons toujours à la micropipette et à l intérieur de la gouttelette un peu d eau pepton e L opération est renouvelée 3 ou 4 fois Ainsi nous avons obtenu sur 10 isolements 3 cultures apparentes dans la chambre à huile

Dissociation

Discussing the problem of the dissociation of the plague bacillus in 1936 Pollitzer[1] pointed out the curious fact that the question of whether this micro organism occurred normally in the smooth (S) or the rough (R) form had been answered in diametrically opposite ways by different observers Several of them particularly Bezsonova & Lenskaia[16 17] considered the rough as the usual and virulent type of the plague bacillus and the same has been maintained by some recent authors such as Bezso nova[14] and Korobkova[99]

Pirie[13] on the other hand distinguished between a normal smooth and a mutant rough type as follows

Normal S type	*Mutant R type*
Bipolar staining round-ended plump bacilli	Long thin bacilli often thready bipolar staining indistinct
Agar colonies small smooth regular convex colourless or faintly bluish	Agar colonies larger often granular rugose yellowish or reddish brown
Growth sticky	Growth not sticky
Homogeneous clouding in broth	Agglutinative sedimentation in broth
Virulent	Non virulent

Observations made by Pirie regarding the behaviour of his strains in salt solutions gave no clear-cut results However Rachinski[140] who on the whole confirmed Pirie s findings when carefully studying the disso ciation of a plague strain noted that his S variant produced a homogeneous suspension in normal saline while the R variant failed to do so

It is of great interest that following a suggestion made by Pollitzer[13] in 1936 Wats & Puduval[189] recently made observations similar to those described above not only on the virulent and avirulent variants of a laboratory strain but also upon primary cultures isolated during a plague epidemic from the blood and buboes of patients The results of their investigations may be summarized thus

Laboratory strain I 0 5H

Virulent form	*Avirulent form*
Organisms showing normal ovoid form	Organisms long and thin exhibiting pleo morphism
Majority of agar colonies small smooth transparent convex glistening with regular margins	Majority of agar colonies fairly large with pin pricked or rugose surface flat opaque with serrated or broken peripheries
Uniformly homogeneous growth in broth	Granular growth in broth with deposit at bottom and sides and fairly clear fluid
Stable in 0 85% saline	Salt sensitive

The two colony types described above were also present in the bubo and blood cultures from plague patients but in the blood cultures an

intermediate form was found in addition consisting of medium sized colonies with irregular margins and a ruffled surface, sometimes showing a nippling in the centre

Wats & Puduval added that

"In colonial characteristics morphology of the organisms, nature of growth in broth salt stability and other characteristics the small smooth colonies resembled those obtained from the virulent cultures of 120/5H and the large whitish 'rough colonies were similar to the ones obtained from the avirulent strain The intermediate type microscopically showed normal type of ovoid organisms as well as marked pleomorphism and as its name implies seemed to be a middle stage between the two colonies"

Observations on the percentage of S and R forms, made by the two workers when subculturing single colonies of virulent and avirulent stock strains on blood agar at room temperature (27° 29° C), and 37° C respectively, are summarized in table IX

TABLE IX PERCENTAGE OF SMOOTH AND ROUGH COLONIES FROM VIRULENT AND AVIRULENT STRAINS OF PLAGUE BACILLI INCUBATED AT 27 29 C AND 37 C

Strains	Percentage of colonies			
	27 29 C		37 C	
	Smooth	Rough	Smooth	Rough
Virulent				
120 5H	85	15	98	[illegible]
V miaba	27	73	65	5
34/B	30	70	78	27
35/B	60	40	80	20
36 H	58	42	55	45
Avirulent				
120/5H	2	98	8	92
P	10	90	12	88
Q	6	94	10	90
55/H	75	25	92	8

It will be noted that generally speaking smooth colonies vastly preponderated in the cultures of virulent plague strains kept at body temperature but were less numerous in the case of room temperature incubation With one *noteworthy exception, the avirulent strains yielded* an overwhelming majority of rough colonies regardless of differences in the incubation temperature

The findings of Wats & Puduval which are in accord with those made by earlier observers such as Pirie [13] and Rachinski [140] not only render it likely that the smooth type is characteristic for naturally occurring or

freshly isolated plague strains but also go a long way towards explaining why so many observers had come to a divergent opinion For it may be maintained that in the laboratory at least the plague bacillus is to some extent invariably in a process of dissociation and therefore apt to respond quickly to any unfavourable environmental influence with reactions suggestive of a transition from the normally smooth to the rough state However changes in extrinsic features such as the morphological aspect character of growth on solid media or in broth and behaviour in salt solutions are by no means necessarily accompanied by changes in essential intrinsic properties such as virulence and immunogenic power Hence, as has been justly claimed by such observers as Otten[1] Girard[6] and Macchiavello,[108] it would be wrong to apply the definitions of dissociation found appropriate in the case of other bacterial genera too rigidly to the case of the plague bacillus which shows in some respects a behaviour of its own

Levine & Garber[10] found addition of triphenyl tetrazolium chloride to tryptose agar in a final concentration of 0 005% useful for a differentiation of smooth and rough forms of *P pestis* The smooth colonies were about 2 mm in diameter and round and had a sharply defined carmine red centre The rough colonies were of an irregular shape and diffusely pink in colour

As described by Garber et al[61] in a subsequent paper identical morphological differences between smooth and rough colonies could be detected when *P pestis* cultures grown on tryptose agar to which 0 2% glucose and 0 8% yeast extract had been added were examined in obliquely transmitted light

Pigment formation

Instances of pigment formation in plague cultures or as Devignat[31] called it of chromogenic dissociation have been described by a few observers—more recently Bezsonova[13] (development of yellow pigmentation in a strain of Indian origin which changed to a brown or pink coloration in subcultures) Korobkova[100] (formation of yellow pigment) and Devignat[31] who in the course of the preparation of live avirulent plague vaccine observed the sudden appearance of a yellowish coloration in his EV strain which dissociated at the same time into rough and smooth variants

A further instance of pigment formation by *P pestis* was recorded in 1952 by Girard[73] who found a reddish pink colony consisting of Gram negative bacilli morphologically identical with *P pestis* on an agar plate seeded about three weeks previously with material from a culture of the avirulent EV strain which had been kept for ten years without subculture Transfer of this colony in bulk after admixture of a drop of plague bacteriophage resulted in pigmented growth the bacilli in question having

afterwards the strain failed to produce acidity in the above mentioned media and as confirmed by Girard [69] displayed in general typical features of plague.

To ascribe the changes in the behaviour of this culture to mutation is impossible for whereas a plague strain might cause different biochemical reactions at different times, it could never prove pathogenic for chickens. Great attention must be paid, therefore, to the suggestion of Girard [69] that probably besides the plague bacillus another micro organism had been present in the original cultures which was responsible for the aberrant reactions but which eventually disappeared. Quite possibly such instances of a mixed infection which might be responsible for unusual clinical and laboratory findings are less rare than is usually assumed.

As Girard [4] admitted himself pending further studies it is impossible to gauge the significance of the experience made with the above mentioned pigment forming EV substrain and that made with another substrain which produced no pigment but also showed evidence suggestive of mutation under the influence of plague bacteriophage.

BIOCHEMICAL PROPERTIES

Alkali production in broth cultures

Notwithstanding some early statements to the contrary, it is by now generally agreed that growth of the plague bacillus in broth results in the production of alkali consisting according to Mayr [117] partly of ammonia. It should be noted, however, that according to some authorities such as Korobkova [9] passing acidity may precede alkali formation.

The following details may be mentioned in this connexion.

Bannerman [6] found a regular increase of alkalinity in broth cultures week by week which reached its maximum corresponding to 1.5%–2% N/NaOH in 6 8 weeks. While further growth ceased at this time, the bacilli were apt to survive in such alkaline media for at least 18 months. Moreover, neutralization resulted in further growth until once more the maximum of alkalinity had been reached, so that the stoppage of growth could not have been due to an exhaustion of the media as was originally assumed by Haffkine [8]. Bannerman thought it best to use slightly acid broth for plague work.

Naidu & Jung [120] repeating such investigations on a larger scale, found that the maximum of alkalinity, followed by a series of small rises and falls was reached between 5 and 11 weeks of growth in broth. Even at the end of the cultivation period of 176 days abundant growth was obtained on agar subcultures.

apparently lost the antigenic and immunizing properties of the parent strain However non pigmented secondary growth showing all the characteristics of the original strain, eventually became manifest on the surface of one of the peptone water-tubes used for subcultivation

Crystal formation

As stated by Girard [74] occasional appearance of crystal needles had been observed by him and by Robic on agar slants seeded with the avirulent EV plague strain It was found afterwards that such crystal formation took place regularly if a 2% agar medium (pH 7) made from fresh beef infusion and containing 20 g Uclaf' peptone besides 5 g NaCl per litre, was used for cultivation of this strain The crystals in question appearing after a cultivation of about two weeks amidst the plague bacilli had usually a white but sometimes a yellowish colour Their appearance seemed to enhance rather than to impair the vitality of the growths in question Subcultures made from these were free from crystals Two other avirulent plague strains as well as a pseudotuberculosis strain grown on the same medium showed no crystal formation

Mutation

As summarized by Pollitzer,[135] up to 1936 no convincing observation of a mutation of the plague bacillus had been recorded, the atypical strains described by a few workers appearing to be variants due to dissociation rather than true mutants The same seems to hold true for some of the more recent observations For instance Macchiavello [108] claimed to have found two plague strains which in his opinion had undergone true and stable mutation but it would seem that these aberrant growths differed from typical plague strains in the degree of their reactions rather than fundamentally

It deserves attention however, that Bezsonova et al [18 19] claimed to have observed five instances of a spontaneous transmutation of plague into pseudotuberculosis bacilli and that Korobkova [89] and Tumansky [18] maintained that they had produced such a transition through bacteriophage action Since as has been mentioned already and will be discussed more fully in chapter 5, opinions as to what tests are valid for a differentiation between plague and pseudotuberculosis bacilli vary considerably great caution has to be exerted in evaluating these claims

Most unusual findings were recently made by Reynes [14] when dealing with a strain which had been isolated from a patient suffering from an atypical form of plague described by Alain & Reynes [1] Soon after isolation this strain not only acidified fluid glycerol and rhamnose media, but also proved pathogenic for chickens However when re examined five years

afterwards the strain failed to produce acidity in the above mentioned media and as confirmed by Girard [69] displayed in general typical features of plague

To ascribe the changes in the behaviour of this culture to mutation is impossible for whereas a plague strain might cause different biochemical reactions at different times it could never prove pathogenic for chickens Great attention must be paid therefore to the suggestion of Girard [69] that probably besides the plague bacillus another micro organism had been present in the original cultures which was responsible for the aberrant reactions but which eventually disappeared Quite possibly such instances of a mixed infection which might be responsible for unusual clinical and laboratory findings are less rare than is usually assumed

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Petrie[123] reported in 1929 on similar work, his data may be summarized as follows

Growth media	Number of strains	Cultivation period (weeks)	Cultivation temperature (C)	pH initial	pH final
Ordinary broth	5	9	6 12	7 3	7 8
Broth with 1% horse serum	53	7	10	7 4	8 4
Ordinary broth	6	8	11 15	7 6	8 0

Otaka,[124] testing different media found the hydrogen ion concentration of the medium increased when meat juice was used for cultivation of plague bacilli and diminished when peptone water was inoculated, growth in ordinary bouillon seemed to represent a balance between these opposite reactions He recommended, for carbohydrate tests particularly a medium the pH of which did not change during cultivation

Nikanoroff[1] making comparative tests with sugar free broth found the pseudotuberculosis bacillus a more rapid and energetic alkali producer than the plague bacillus and as will be discussed later, recommended special media to demonstrate this difference between the two micro organisms

Action on milk

Most workers maintain that plague bacilli do not curdle milk and that they, as well as the pasteurellae sensu stricto fail to produce alkalinity *in litmus milk* *In view of the fact that generally speaking, the pseudo* tuberculosis bacilli turn litmus milk alkaline, it was recommended to use this medium for differential diagnostic purposes However, as pointed out by Nikanoroff,[122] the value of this and other tests based upon changes in reaction of the media is not absolute because occasionally poor alkali producers are encountered among the pseudotuberculosis strains Aberrant *reactions have been observed in case of the plague bacillus as well*

Hydrogen sulfide and indole production

General agreement exists that both plague and pseudotuberculosis bacilli fail to produce hydrogen sulfide in the course of cultivation, while the pasteurellae sensu stricto do so

The difference displayed by these micro organisms in regard to the production of indole is not so absolute for whereas plague and pseudo tuberculosis bacilli invariably prove negative in this respect and most of the other pasteurellae positive Schutze[159] stated that a few of the latter also fail to produce indole

Reduction of nitrates

Observations regarding the ability of the above mentioned micro-organisms to reduce the nitrates contained in the media to nitrites,

eventually producing nitrous acid gave divergent results Schutze[1 9] dealing with the pasteurellae sensu stricto stated that ' nitrates are reduced to nitrites , but Henriques[8] found that the strains examined by him did not produce this reaction Further, although the pseudotuberculosis bacilli usually prove negative in this respect some exceptions to this rule noted by Girard[64] and by Konovalova[96] will be discussed later

Testing plague cultures for the presence of nitrites Gore[9] invariably got positive results His experiences were confirmed by Micheletti[119] who noted that even in ordinary bouillon a certain amount of nitrous acid was produced by the plague bacillus and more recently by Petragnani[1 3] who considered tests for the presence of nitrous acid made with cultures grown in liver bouillon for 24 48 hours as one of the cardinal methods for differentiation between plague and pseudotuberculosis bacilli Devignat & Chevalier[39] on account of systematic studies carried out with media which were practically or even chemically free from nitrates fully confirmed that strains of *P pestis* were capable in part of producing nitrites from proteins present in these media

Petragnani s claim that apart from the plague bacillus only cholera vibrios gave positive reactions when tested in this manner was not confirmed by Henriques[8] who obtained identical results with *Shigella* and *Alcaligenes faecalis* (*Bacillus faecalis alcaligenes*) strains Devignat[37] thus listed the microorganisms which could produce nitrites in nitrate free broth media

Strongly positive	*Slightly positive*
Pasteurella pestis	*Aerobacter aerogenes*
Alcaligenes faecalis	*Hemophilus pertussis*
Corynebacterium diphtheriae	*Klebsiella pneumoniae*
Hemophilus influenzae	*Moraxella lwoffi*
Moraxella duplex	*Mycobacterium paratuberculosis*
Vibrio comma	*Proteus morganii*
Vibrio fetus	*Proteus rettgeri*

Note In contrast to Henriques[8] Devignat did not obtain positive results with *Shigella dysenteriae* and allied species

The validity of the nitrite reaction for differentiating plague and pseudotuberculosis bacilli was doubted by Fusco[57] and by Girard[64] The former maintained that not only plague but also pseudotuberculosis bacilli gave a positive reaction for nitrites when 24 hour broth cultures (or the condensation water of agar cultures) were tested with the reagent of Griess Girard found that one out of eight pseudotuberculosis strains caused a slight reduction from nitrates to nitrites Out of his 103 plague strains practically all of which seem to have been isolated in Madagascar 90 gave a positive reaction with the reagent of Griess which however varied considerably in intensity Four strains which appeared to be

negative at first, also showed the presence of nitrites when re examined three months later, but nine strains (five of which were virulent) proved constantly negative Girard concluded therefore, that tests for nitrites were not as valuable for the differentiation of plague and pseudotuberculosis bacilli as those with rhamnose

It deserves great attention, however, that, as shown by the experiences of Konovalova [96] and of Devignat,[35, 36] racial differences exist among the *plague strains isolated in different areas*

Konovalova [96] tested a large number of plague strains as well as some pseudotuberculosis strains for nitrites, with the following results

Media used	*Plague strains positive*	*Plague strains negative*	*Pseudotuberculosis strains positive*	*Pseudotuberculosis strains negative*
Nitrate free ordinary broth	6	140	2	8
Same broth with 0 1% potassium *nitrate*	*9*	*137*	*10*	—

Commenting upon these findings, Konovalova stated that all Indian Italian, and Transbaikalian plague strains at her disposal gave a positive *result when grown in broth to which potassium nitrate had been added,* while, with two doubtful exceptions, all the strains from south east Russia proved negative In nitrate free bouillon also positive reactions were given only by strains received from abroad, including all three Indian strains

Since the classification of the plague bacillus into races postulated by Devignat [35 36] is based upon fermentation tests as well as upon such tests for the presence of nitrites his findings will have to be discussed later

Enzyme activity

(*a*) *Catalase and ribonuclease* Herbert [86] found that whenever the plague strains tested by him could be induced to grow in his amino acids glucose medium or on agar plates they produced catalase, even if no haemin had been added to the media Considering catalase to be a haematin protein Herbert concluded, therefore, that the plague bacillus was able to synthesize haematin

Investigating the relation between catalase activity and virulence of the plague bacillus Rockenmacher [146] found 14 virulent strains significantly more active in decomposing hydrogen peroxide than 11 avirulent strains and *suggested, therefore that tests measuring the* catalase activity might be used for screening the virulence of plague strains in vitro

The ribonuclease activity of *P pestis* was studied by Woodward [194] who with the aid of hydrochloric acid precipitation and uranium fractionation, was able to show that the ribonucleic acid of yeast was enzymatically decomposed by living plague bacilli as well as by those killed with phenyl mercuric acid and by preparations free from bacterial cells

(*b*) *Fibrinolysins* Carrying out tests with serum free fibrin clots Madison[11] found that the plague bacillus showed marked fibrinolytic activity particularly for rat and guinea pig fibrin

(*c*) *Coagulase* Jawetz & Meyer[89] while unable to confirm that *P pestis* had fibrinolytic powers established with the aid of Fisk s method that both virulent and avirulent plague strains showed a coagulase activity However only rabbit and guinea pig plasma was coagulated and not that of other species including man

(*d*) *Urease* It may conveniently be added that as recently established by Fauconnier[50] 24 pseudotuberculosis strains of human or mammalian origin as well as 7 out of 9 such strains of avian origin produced rapid hydrolysis of urea on the synthetic medium of Ferguson or on Roland Bourbon s urea tryptophane medium With the exception of the above mentioned atypical probably impure strain of Reynes[114] the 35 plague strains tested showed no urease activity Fauconnier therefore recommended this method for differentiation of plague and pseudotuberculosis bacilli

Action on carbohydrate substances

The action exerted by the plague bacillus on sugars or allied carbohydrate substances has been studied by numerous investigators with the result that the micro organism was found capable of producing acidity but no gas in media containing certain of these substances leaving others unchanged Details of these findings as well as results obtained with the pseudotuberculosis bacillus and the pasteurellae sensu stricto are set forth in table X which is based upon a summary of the literature available up to 1936 by Pollitzer[135] as well as upon recent records especially those of Devignat & Boivin[38] Francis[44, 50] Henriques[80] Matumoto[115] Prado[134, 139] and Russo[151, 15]

Differential diagnosis Though a considerable amount of early work had been done with carbohydrate-containing media it was only in 1926 that Colas Belcour[0] made a definite proposal to use them for differential diagnostic purposes He then recommended glycerol litmus agar to distinguish between plague and pseudotuberculosis bacilli supposing that the former produced no change in this medium while pseudotuberculosis strains invariably acidified it However as suggested by some observations made before 1926 and confirmed by ample further investigations glycerol containing media are unsuitable for this purpose in view of the frequent occurrence of plague strains which like the pseudotuberculosis bacillus produce an acid reaction in such substrates

A recommendation to use media containing rhamnose instead of glycerol media for the differentiation of the two organisms was made by Bezsonova[11, 1] It has to be noted in this connexion that without any well documented exception pseudotuberculosis bacilli rapidly acidify rhamnose

TABLE X ACTION OF VARIOUS PASTEURELLAE ON CARBOHYDRATE SUBSTANCES

Carbohydrate substance	*P pestis*	*P pseudo tuberculosis*	Pasteurellae sensu stricto
Adonitol	±	+ *a*	—
Amygdalin	—	±	— *a*
Arabinose	+ *a*	+	— *a*
Dextrin	±	±	—
Dulcitol	± *d*	— *a*	— *a*
Erythritol	—	—	—
Galactose	±	+	+
Glucose	+	+	+
Glycerol	±	+	—
Glycogen	+ *a*	— *b*	—
Inos tol	—	—	—
Inulin	— *a*	— *a*	—
Lactose	— *b*	—	—
Laevulose	+	+	+ *a*
Maltose	±	+	— *a*
Mannitol	+ *c*	+	+ *a*
Mannose	+	+	+
Melibiose	—	+	—
Raffinose	—	±	—
Rhamnose	— *b e*	+	—
Saccharose	— *b*	±	+ *a*
Salicin	±	+ *a*	— *a*
Sorbitol	— *e*	±	+
Starch	±	±	—
Trehalose	± *d*	+	±
Xylose	±	+	±

\+ = acidification — = no acidification ± = positive or negative

a Some exceptions were noted
b Late acidification was observed in some cases
c Pons found only two thirds of his freshly isolated strains positive
d Negative reactions were more common than positive ones
e One slightly positive reaction noted by Devignat & Boivin[38]

containing media while plague bacilli almost invariably fail to do so though they sometimes produce late acidification Therefore the value of this method has been endorsed by several observers quite recently and emphatically by Girard [0]

Russo [151 15] and Prado [138 139] drew attention to two other substances—melibiose, which in their opinion was specifically attacked by the pseudo tuberculosis bacillus and glycogen the acidification of which they considered as almost characteristic for the plague bacillus Matumoto [115] who

repeated tests with glycogen only found that the plague bacillus gave inconstant reactions in media containing it. It would be most desirable not only to follow up this matter and to make further tests with melibiose but also to re-examine in general and in a thorough manner, the behaviour of the plague bacillus and the micro-organisms more or less resembling it in media containing sugars and allied carbohydrate substances.

Though a few claims to the contrary have been made, the bulk of the available evidence shows that generally speaking the reactions produced by individual strains of the plague and pseudotuberculosis bacillus in carbohydrate-containing media were stable in character. This contention is well illustrated by the observations of Francis[61, 5] who found that a plague strain, when re-examined after 20 years and again 5 years later, behaved in such media as it had done originally.

While in agreement with the general rule set forth above no convincing evidence exists that the reactions produced by plague bacilli in media containing glycerol are subject to changes (Chen[29]), the problem of the behaviour of these micro-organisms in rhamnose media is far less unequivocal.

Bezsonova et al.[18, 19] stated they had found five plague strains which, though at first inert in rhamnose media, became capable of acidifying them after having been kept in the laboratory for years. However, as noted above, Bezsonova and her co-workers ascribed this change to a transmutation of the strains into pseudotuberculosis bacilli.

Korobkova[99] claimed to have obtained rhamnose-positive variants of *P. pestis* through bacteriophage action. Similarly it was reported by Berlin & Borzenkov[9] and also by Tumansky,[184] that plague strains kept in liquid media containing rhamnose were apt to dissociate into two variants, one of which was capable of producing acidity when grown in the presence of rhamnose. Krainova[10] claimed in this connexion that only glycerol-positive strains from Manchuria, Mongolia and south-east Russia could acquire this property, the glycerol-negative cultures obtained from India and Italy as well as Girard & Robic's EV strain remaining unaltered when subjected to passage through rhamnose media. She assumed therefore that the Eurasian strains were more closely related to the pseudotuberculosis bacillus than those obtained from other parts of the world.

Action on glycerol for race differentiation

Though, as stated above, tests with glycerol-containing media are not useful for differential diagnostic work, they deserve, as first established by Bezsonova,[10] great attention for another reason. Noting that the plague strains isolated in south-east Russia, Transbaikalia and Turkestan invariably acidified glycerol media whereas some strains from India and one from Italy failed to do so, she considered it likely that glycerol-positive and glycerol-negative strains did not exist side by side, only one kind of strain occurring in any given area.

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Arabinose	+ a	+	— a
Dextrin	±	±	—
Dulcitol	± d	— a	— a
Erythritol	—	—	—
Galactose	±	+	+
Glucose	+	+	+
Glycerol	±	+	—
Glycogen	+ a	— b	—
Inositol	—	—	—
Inulin	— a	— a	—
Lactose	— b	—	—
Laevulose	+	+	+ a
Maltose	±	+	— a
Mannitol	+ c	+	+ a
Mannose	+	+	+
Melibiose	—	+	—
Raffinose	—	±	—
Rhamnose	— b e	+	—
Saccharose	— b	±	+ a
Salicin	±	+ a	— a
Sorbitol	— e	±	+
Starch	±	±	—
Trehalose	± d	+	±
Xylose	±	+	±

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Dextrin	±	±	—
Dulcitol	± *d*	— *a*	— *a*
Erythritol	—	—	—
Galactose	±	+	+
Glucose	+	+	+
Glycerol	±	+	—
Glycogen	+ *a*	— *b*	—
Inositol	—	—	—
Inulin	— *a*	— *a*	—
Lactose	— *b*	—	—
Laevulose	+	+	+ *a*
Maltose	±	+	— *a*
Mannitol	+ *c*	+	+ *a*
Mannose	+	+	+
Melibiose	—	+	—
Raffinose	—	±	—
Rhamnose	— *b e*	+	—
Saccharose	— *b*	±	+ *a*
Salicin	±	+ *a*	— *a*
Sorbitol	— *e*	±	+
Starch	±	±	—
Trehalose	± *d*	+	±
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Though as stated above tests with glycerol containing media are not useful for differential diagnostic work they deserve as first established by Bezsonova[10] great attention for another reason Noting that the plague strains isolated in south east Russia Transbaikalia and Turkestan invariably acidified glycerol media whereas some strains from India and one from Italy failed to do so she considered it likely that glycerol positive and glycerol negative strains did not exist side by side only one kind of strain occurring in any given area

Amplifying this idea, Kurauchi[104] and Berlin & Borzenkov[9] distinguished between two races of plague bacilli—a glycerol positive continental race characteristic according to Berlin & Borzenkov, in the first line of the central Asiatic plateau and adjacent territories, and an oceanic race found in the peninsulas and islands of the tropics into which plague had been introduced by the sea route Berlin & Borzenkov also assumed the existence of transitional areas where both races might occur Chen[8] and Matumoto[116] expressed agreement with these views

As shown by table XI, which embodies the results obtained by the above mentioned as well as other observers, no doubt can exist that the plague strains isolated in Manchuria, Mongolia south east Russia Trans baikalia and Turkestan produced always or practically always an acid reaction in glycerol media Less ample evidence tends to show that a second area where glycerol positive strains prevail or are even present singly exists in the Belgian Congo Uganda and part of Kenya Thus it would seem that the presence of glycerol positive strains is characteristic for territories where plague has existed since time immemorial—as in Central Asia and the adjacent areas—or is at least of very long standing as we have much reason to assume is the case in Central Africa At the same time the prevalence of glycerol acidifying strains in the latter area goes a long way towards confirming Payne s hypothesis mentioned in chapter I that the African was a colony of the Asiatic plague

It appears on the other hand that as postulated by Berlin & Borzenkov,[9] glycerol negative strains prevail or are even found singly in the countries which were infected —mainly by the sea route—during the present pandemic The fact that the strains isolated in California fall into this category speaks against the assumption that the origin of plague there is connected with an ancient immigration of wild rodents from Asia

That one of the transitional ' areas presupposed by Berlin & Bor zenkov[9] might be located in the Union of South Africa is rendered likely by the observations of Pirie[131] who found in one isolated focus of Cape Province four glycerol positive strains while the 19 strains from other local foci examined by him did not alter the reaction of glycerol media

The occurrence of both glycerol positive and glycerol negative strains was also noted in Japan and in the Philippines Details furnished for the former country by Matumoto[116] may be summarized thus

Locality	*Year*	*Number of strains examined*	*Glycerol* negative	*Glycerol* positive
Yokohama	1913	4	0	4
Tokyo	1914	5	0	5
Osaka	1922	9	9	0
Yokohama	1926	1	1	0
Osaka	1929	2	2	0

TABLE XI BEHAVIOUR OF PASTEURELLA PESTIS IN GLYCEROL MEDIA

Local ty	Number of stra ns examined	Acidifi at on of glycerol		B bliographical reference
		Negative	Positive	
Arabia	1	1	0	31
Argent na	50	40	2	18
Brazil	45	45	0	108 1 1 15?
Cal fornia	6	65	0	29 31 41 53 101
Ceylon	7	7	0	130
Ch na (Fukien)	2	?	0	108
Belg an Congo	43	0	43	6
Egypt	3	3	0	9
England	35	3	0	46
Formosa	2	?	0	94 103
France	1	1	0	30
French West Africa	4	4	0	137
Germany (Hamburg)	1	1	0	53
Greece	1	1	0	30
Hawai	10	10	0	28
Ind a	20 [b]	20	0	10 28 31 130 151 152 188
Indoch na		1	1 [c]	30 145
Iran (Kurd stan)	3	0	73	5
Italy		2	0	10 53
Japan	33	3	10	94 103 104 116
Java	8	7	1 [d]	28 116
Kenya	69	53	16	163 194
Madag scar	6	6	0	28 30 6? 75
Manchuria	84 [f]	83	1 [g]	29 31 94 103 104 116
Mongol a	20	0	20	9 94
Pale t ne	2	2	0	[illegible]
Ph l ppines	11	7	4	31 107
South Afr ca	25	21	4	8 131
Sou h-ea t Russia	167	0	167	10 15 93
Southern USA	9	26	3	31 187
Sumatra	1	1	0	177
Tha land	1	0	1 [h]	54
Transba kal a	6	0	6	10
Turkestan	22	0	22	10 15
Turkey	4	3	1 [i]	193
Total	855	480	375	

[a] According to Devignat the plague strains isolated in Uganda also invariably acidified glycerol media

[b] It is of great interest that out of the 12 Indian strains recen ly examined by Wagle & Habbu four had been isolated in North Ind a—one in Uttar Pradesh and three in Bettiah Bihar not far from the Nepal border

As stated in the text (page 97) the glycerol positive strain of Reynes was probably impure

[d] The Java strain found positive by Chen was stated to be a subculture of Otten s avirulent Tjiwidej stra n which usually does not acidify glycerol med a

Girard repeatedly stated that all plague strains isolated in Madagascar proved negat ve on glycerol and rhamnose med a

[f] Included are 13 strains from Vladivostok and neighbouring places which had become secondarily invaded during the 1921 pneumonic plague ep demic

[g] This was a strain in the series of D Aunoy sa d to have come from Manchuria

[h] Francis was not certain that the glycerol pos t ve stra n forwarded to Washington from Thailand had actually been isolated in that country

[i] This glyce ol acidifying strain had been isolated during the 1947 ep dem c at Akçakale on the Syrian bo der whereas the three other st a ns examined in Tu key came from po ts

Matumoto drew attention to the fact that strains isolated during the same outbreak all showed a uniform behaviour in glycerol media and also suggested, with much reason that Japan had been reached by two waves of the infection which seemed to have been imported first from the north, possibly from Manchuria, and then from the south

As suggested by Devignat,[36] a similar state of affairs might have existed in Kenya where, in contrast to the findings made in the Belgian Congo and Uganda, de Smidt [168] recorded the occurrence of glycerol negative strains Since however, plague had been imported into Kenya by the sea route before these findings were made, Devignat considered it likely that the manifestations of the disease during which the strains in question had been isolated, resulted from this sea borne infection This assumption has been fully confirmed by the recent observations of Heisch,[84] which showed that all plague strains isolated thus far in the Rongai area of Kenya acidified glycerol media

As will be gathered from the observations discussed above a striking contrast exists between the glycerol reactions of the plague strains isolated in the original foci of the infection in Central Asia and those found in most of the secondarily invaded areas throughout the world No doubt a change in the behaviour of the strains towards glycerol must have taken place during the spread of the infection from Central Asia but it is difficult to determine where and how this transition took place Owing to the lack of actual observations, it can be merely inferred that the plague strains in Burma are glycerol negative It is nevertheless, in the opinion of the present writer most likely that the change took place when the infection passing from the wild rodent foci in Central Asia to Burma, became entrenched among *Rattus rattus*, which has its original home in that country and in Assam [c]

This assumption is not contradicted by the fact that the plague strains in Uganda and in the Congo did not alter their behaviour towards glycerol because as noted before, in these areas the infection at first became entrenched not among the usual species of commensal rats, but among *R natalensis (Mastomys coucha)*

Although as pointed out by Berlin & Borzenkov,[9] glycerol negative plague strains belonging to the oceanic race were responsible for the manifestations of the disease due to recent importations of the infection by the sea route there can be no doubt that the outbreaks taking place during the Black Death were caused by glycerol positive strains and the same probably held true of the pandemic in Justinian s time which seems to have originated in Central Africa

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[c] It is interesting to note in this connexion the belief expressed by G rard [*] that regardless of geographical di tribution acidifi ation of glycerol media was peculiar to plague strains from wild rodents while growths of rat or rat flea origin produced no acidity However as pointed out by Chen this assumpt on could not be regarded as generally valid because for instance the plague strains isolated in California e en if of direct wild rodent origin proved negative in glycerol media

Bearing in mind the fact that pneumonic plague was rampant during the Black Death and also that the devastating pneumonic plague outbreaks in Manchuria (1910 11 1920 1) as well as that in Shansi (1917 18) were caused by glycerol positive strains, it would be tempting to assume that the continental race of the plague bacillus might be particularly apt to produce lung involvement It must be noted however, that pneumonic features were by no means invariably conspicuous in outbreaks caused by this race of *P pestis* and that, on the other hand the incidence of pneumonic plague was conspicuous in areas where only glycerol negative strains were present for instance in Madagascar Generally speaking although the differentiation of the plague bacilli with the aid of glycerol tests is valuable for the solution of nosogeographical problems thus far no convincing evidence exists of any fundamental difference between glycerol positive and glycerol negative strains

Considering the results of tests not only in glycerol media but also those obtained with the aid of rhamnose Kawashima[91] came to the conclusion that the plague bacilli might be divided into three groups strains acidifying neither substance those producing acid in both and finally those positive in glycerol media only Since according to the observations of the Russian workers quoted above tests with rhamnose might yield less stable results than those with glycerol it does not seem advisable to use the former for purposes of classification It should be noted moreover that Chen [8] found two plague strains not falling into Kawashima s scheme because they produced late acidification in rhamnose media though no change in those containing glycerol

It is however of great interest that Devignat [3] [36] recently proposed to classify the plague bacilli as follows

Proposed subgroups	*Tests with glycerol*	*Production of nitrous acid*	*Remarks*
P pestis			
var *orientalis*	−	+	Corresponding to Berlin & Borzenkov s[8] oceanic race
var *antiqua*	+	+	Found in Central and North Asia and in Central Africa
var *mediaevalis*	+	−	Found in south-east Russia

Devignat was of the opinion that the var *mediaevalis* of the plague bacillus was capable of becoming transmuted into pseudotuberculosis bacilli which also acidified glycerol media but did not produce nitrous acid

Seeing that Girard [63] [64] [70] expressed serious misgivings regarding the reliability of tests showing a reduction of nitrates to nitrites or production of nitrous acid further studies would seem necessary before Devignat s classification could be accepted

VITAL RESISTANCE

It is significant that the bulk of the enormous literature dealing with the behaviour of the plague bacillus outside the living organism dates back to the first years after the discovery of this bacillus, when the time honoured idea that contaminated objects, the so called 'fomites', were of great epidemiological importance still loomed large in the minds of workers. Further research established that the role of contaminated objects in the propagation of plague was rather limited, infection as a rule being insect borne, especially flea borne, or—in the case of the pneumonic type—spreading directly from man to man. In many ways, therefore, the earlier studies on the vital resistance of the plague bacillus may now be said to possess academic rather than practical interest. Further, numerous though observations of this kind are, their results are but rarely comparable or generally applicable. This is due not only to the different extrinsic conditions, such as light, temperature and humidity, under which the work was done, but also to differences in the objects examined. It is probable that when pure cultures were tested, those of long standing, adapted to a life on artificial media, were more resistant than freshly isolated growths. On the other hand, no doubt can exist that in its natural environment the plague bacillus is often better protected than in plain fluids or culture media and is hence less amenable to harmful influences. Ample evidence for this is furnished by tests with material such as pneumonic plague sputum, and by observations upon the prolonged survival of the bacilli in dead bodies and carcasses of infected animals,[d] as well as by comparative tests in vitro (Schiavone & Trerotoli,[156] Galeotti,[60] Wright[19])

Wright ascribed the rapid destruction of plague bacilli unprotected by the addition of substances such as blood serum or gelatin in watery solutions to features suggestive of plasmolysis and thought that this adverse effect might be counteracted by adjusting the ion balance of the fluids.

In fact, Girard[66] found that at temperatures not exceeding 25° C plague bacilli could be kept alive and virulent in normal saline for periods up to one week. At low temperatures (2°-4° C) the bacilli could survive in normal saline for up to two years, remaining pathogenic for intracutaneously infected guinea pigs for periods up to 220 days and for intraperitoneally infected mice up to 685 days.

Resistance to physical and chemical agents

Data of practical importance concerning the vital resistance of the plague bacillus to various physical and chemical agents may be summarized thus:

(a) *Sunlight.* While generally speaking the plague bacillus is easily killed by sunlight, the length of time required for this varies within quite

[d] Recent observations confirming the prolonged survival of plague bacilli in the bone marrow of infected animals in particular were recorded by Uriarte & Morales Villazón [illegible] and by Russo.[illegible] However, Macchiavello & Paracampos[illegible] obtained far less satisfactory results in the hot climate of Brazil.

considerable limits according to the kind of vehicle and the thickness of the layer used the kind of material upon which the infected matter is placed and the temperature

Thus as summarized by Pollitzer[11] one hour s exposure to the sun was sufficient to kill plague bacilli spread in thin layers on coverglasses but they survived for up to 4 hours when exposed in the same manner in thick layers The bacilli contained in thin layers of pneumonic plague sputum were killed by sunlight in 2 5 hours but at temperatures below 12 C it took up to 12 hours exposure to sunlight to sterilize pneumonic plague sputum expectorated into sterile dishes According to Toyoda & Yasuda[180] this period was prolonged to 14 hours if hempen material instead of glass was used as substratum

(b) *Dry and moist heat* Dry heat was stated to be not very effective against the plague bacillus for exposure at 100° C was found to be necessary for periods up to one hour to ensure killing of the organisms Lower temperatures administered for the same interval of time often proved ineffective

While it is agreed that plague bacilli on instruments or on other inanimate objects are almost immediately killed if the latter are immersed in boiling water opinions as to the length of time necessary to ensure sterility at lower temperatures under the conditions used for vaccine preparation vary considerably Whereas it is now the standard practice of the Haffkine Institute to sterilize the brews through an exposure at 55° C for 15 minutes earlier workers recommended keeping the vaccine flasks in the water bath for at least one hour at 65° C Presumably however insufficient care was taken by the earlier observers to ensure an adequate temperature inside the flasks

(c) *Cold* As confirmed by numerous observations the plague bacillus is not sensitive to the action of low temperatures Slow growth continues to take place when plague cultures are kept in the refrigerator at a temperature of 4° ± 2° C (Sokhey[171]) and Kasinski[9] even found that agar cultures kept for 5½ months at −31° C remained viable though showing some loss of virulence Indeed it may be maintained that low temperatures are beneficial for the development of the plague bacillus because they inhibit the growth of concomitant bacteria which is often inimical to it

It should be noted in this connexion that Shurupoff[148] recovered virulent plague bacilli from the dead bodies of 6 out of 17 pneumonic plague victims who had been interred in a soil rich in salt for periods up to one year having become frozen during the winter Francis[53] keeping the whole spleen of a plague infected guinea pig at −15°C obtained virulent growth from it after 7 years

(d) *Desiccation* Though generally speaking desiccation particularly if taking place rapidly at high temperatures is harmful to the plague bacillus this rule does not hold good under all circumstances

Experience has shown that plague bacilli in sputum or similar vehicles where they are protected by mucus or proteins may withstand exsiccation for considerable periods, especially if placed on substrates like cotton wool and similar materials which in their turn offer some protection to the bacilli

It is interesting to note in this connexion that Pollitzer[135] was able to obtain positive cultures from plague sputum which seemed to have become quite dry and that Nikanoroff[121] found that such dried sputum remained virulent for periods up to 165 days

Uriarte & Morales Villazon[186] established that the viability and virulence of plague strains could be maintained without subculturing for *more than two years if the original growths were kept in vacuo over phos*phorus pentoxide Reitano[144] found that cultures thus dried and kept remained viable for 30 months, but noted that they had become avirulent and non immunogenic However, as established by recent experiences rapid drying from the frozen state is a suitable means of preserving plague cultures for laboratory work Seal & Habbu[163] obtained satisfactory results with this method by adding 5% of gum acacia to broth cultures, 50% of the organisms surviving and remaining virulent for periods of at least 7 months

The question of how long cereals remain apt to harbour plague bacilli has been studied by a few investigators, more recently by Ori[1 3] and Semikoz & Achourova[164]

Ori established that plague bacilli when sheltered from light could remain virulent on maize for 54 days However contaminated maize placed in the centre of a large quantity of the grain in the hold of a ship proved virulent for only 14 days Effective sterilization could be carried out by keeping the maize in a drying-chamber for 10 minutes at 70°C

According to Semikoz & Achourova grain contaminated slightly with organs blood and faeces of plague infected mice became innocuous when kept for 1 8 days under conditions corresponding to those in elevators If the grain was more heavily contaminated and humid faeces were admixed storage in the elevator for 27 days seemed necessary

(*e*) *Disinfectants* While plague bacilli when tested in pure culture, show little resistance to disinfectants in the usual concentrations, they are apt to be less vulnerable when contained in substrates which may retard the action of the disinfectants or neutralize their effect

This point is well illustrated by a series of tests made during the 1920 1 *pneumonic epidemic when the following concentrations were found neces*sary to ensure sterilization of sputum samples (room temperature of 17° C)

Disinfectant	*Concentration*	*Minutes required for sterilization*
Concentrated alcohol (or methylated spirit)	—	4
Carbolic acid	1 10	5
Lysol	1 50	20
Milk of lime	1 10	20
Mercuric chloride	1 500	20
Mercuric chloride	1 1 000	30

Plague sputum was exposed in open Petri dishes in rooms fumigated with sulfur or formalin. Negative bacteriological results were invariably obtained after 12 hours exposure to sulfur fumes. In three experiments the plague bacilli survived 24 hours exposure to formalin.

It has to be added that calcium chloride, though found to be fairly effective in the case of plague cultures, failed to sterilize pneumonic plague sputum even if left to act for 24 hours in a concentration of 20% (Breininger[2]).

It would be of interest to test plague sputum with modern disinfectants, some of which displayed a remarkably strong action upon plague cultures. This holds particularly true of the mercury-phenol compounds tested by Caius and co-workers, quoted by Pollitzer[13]. Graham[79] reported in this connexion that p-chloromercurophenol destroyed in vitro plague bacilli in a dilution of 1 : 400 000 000 in 24 hours. It should also be noted that Skorodumoff & Mitchurina[167] found gentian violet (pyoktanin) and ethoxydiamino-acridine lactate (rivanol) bactericidal when tested with plague cultures in dilutions of 0.01% and 0.02%.

(*f*) *Fixation and staining.* Considering the statements made above it is not surprising to find that the methods usually employed for the fixation and staining of smears prepared from plague material are by no means invariably apt to ensure the killing of the micro-organisms (Jettmar[91], Berdnikov[7], Tinker & Rudnev[1 8]). It is noteworthy that alcohol fixation, which, as will be discussed later, is in general preferable for the preparation of plague smears, gave markedly better results than the usual method of fixation over a flame.

Vitality of cultures

Whereas constant incubation at 37° C is inimical to prolonged life of plague growths, such cultures may remain alive for months if kept at temperatures below 20° C and even for many years if stored in the refrigerator. Most remarkable findings made in this respect by Francis[54 55] when keeping numerous first agar subcultures of a ground-squirrel strain at 5-10° C may be summarized thus:

Period of storage	*Survival*	*Virulence*
20 years	33 of the 48 originally preserved slants gave growth on subculture	22 of the 33 subcultures proved virulent, killing subcutaneously infected guinea-pigs within a week
25 years	25 of the 33 originally preserved slants found viable 5 years previously gave growth on subculture	All 25 subcultures caused acute illness in subcutaneously infected guinea-pigs, but only three of them died of plague within 8 days

That most of the slants tested after 20 years still proved virulent and that virulence still existed to some extent even after 25 years was no doubt due to the fact that the originally preserved growths had been kept without subcultivation which exerts so unfavourable an influence in this respect that it can be used as a means of producing avirulent strains

A classical observation showing the unfavourable influence of subcultivation upon virulence was recorded by Francis [53] a sealed agar culture of a strain of ground squirrel origin kept untouched for 9 years at 10° C proved viable and fully virulent while the same strain when subcultivated every three months throughout this period became *avirulent*

Influence of symbionts

Though as has been noted before, a few bacterial species facilitate the growth of the plague bacillus from small inocula in general its development is much hampered by the presence of other micro organisms

Faddeeva & Tshernobaev [49] who made a systematic study of this matter distinguished between bacteria antagonizing the growth of the plague bacillus and those permitting or even favouring it as follows

Antagonistic	*Symbiotic*
Streptococcus mucosus	*Sarcina*
Pneumococcus	*Staphylococcus aureus*
Coliform typhoid group	*Vibrio paracholerae*
Alcaligenes faecalis	*Klebsiella (Bacillus) o aenae*
(Bacillus faecalis alcaligenes)	*Bacillus aromaticus*
Chromobacterium prodigiosum	*Bacillus subtilis*
Pseudomonas pyocyanea	
Vibrio cholerae	
Bacillus mesentericus	

Dysentery bacilli were partly antagonistic partly symbiotic

While it must be admitted that the bacteria enumerated in the first column above, and also some others such as *B proteus vulgaris* (Tumansky [183]), hamper the growth of the plague bacillus, it is an open question to what extent this phenomenon is due to a specific antagonism of the micro organisms in question or of their products of metabolism and not merely to the comparatively slow and non luxuriant growth of *P pestis* which as it were, is crowded out of existence or at least of demonstrability It is noteworthy in this connexion that, as shown by Jettmar,[90] co existence of plague and tuberculosis bacilli was not harmful to either of the two organisms in vitro or experimentally On the other hand it is important to note that, as recently recorded by Girard [71] rats infected with *Myco bacterium lepraemurium* (Stefansky s Bazillus der Rattenlepra ") showed a marked resistance to experimental infection with *P pestis* Girard [7] also adduced evidence suggesting that human sufferers from advanced leprosy were markedly resistant to plague

Though as shown above the plague bacillus may, under especially favourable conditions persist for even longer periods of time outside the living organism such observations do not invalidate the general rule that this bacillus has feeble powers of resistance to adverse extrinsic influences and is therefore not well adapted to a saprophytic life (Petrie[1, 9]) More over it must be emphasized that though inanimate objects may become and remain for some time infected the chances of their proving infective leading to a spread of the disease are usually remote

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Chapter 3

PROBLEMS IN IMMUNOLOGY

VIRULENCE OF THE PLAGUE BACILLUS

Since immunological terms like "virulence", "virulent" or "avirulent" are often used rather indiscriminately with reference to bacilli, it is necessary to begin the present disquisition by defining in what sense they are applied here. In doing so the apt distinction drawn by Jawetz & Meyer[8] between virulent and avirulent plague bacilli may be quoted:

By a virulent plague bacillus we mean one that will multiply greatly and finally bring about the death of a given susceptible host animal when introduced in numbers not large enough to be toxic without multiplication. An avirulent plague bacillus may then be defined as one not being able to cause the death of the same susceptible host animal unless introduced in numbers sufficient to produce toxic death without multiplication.

Thus, as these authors point out, mere invasiveness cannot be made the criterion of virulence, this term indicating the capacity of the organisms *to multiply in the tissues of the host to a dangerous extent.* At the same time a sharp distinction has to be made between virulence and toxicity. Since, as justly maintained by Jawetz & Meyer, "death from plague in final analysis is always due to a toxaemia produced by decomposition products of the plague bacillus", one might consider the virulence of *Pasteurella pestis* as the means of bringing about a fatal termination of the disease which, however, is ultimately due to the toxicity of the organisms.

Generally speaking, the virulence of the plague bacillus is high. In a classical experiment Barber[8] was able to show that six out of nine guinea pigs and two out of twelve monkeys which had been inoculated with single plague bacilli succumbed to the infection. Identical results have been obtained with guinea pigs by Otten[1-9] while, as will be discussed below, infective doses consisting of not more than six to twelve plague bacilli have been found suitable for standard virulence tests with mice.

It may be further maintained that on the whole the virulence of *P. pestis* is remarkably stable. Petrie[138] summarizing the data of several workers

found that out of 152 laboratory strains of diverse origin, some of them many years old, 12 only showed marked impairment or absence of virulence while the remaining 92% were virulent The interesting experiments of Francis with a 20 year-old plague strain, referred to previously (see page 108) serve as a corollary for these observations

The fact remains, however, that not only may the virulence of plague cultures be abated by artificial means but the cultures may become avirulent spontaneously sometimes even without being kept in the laboratory for prolonged periods (Revenstorf[151])

It has been claimed also by a few observers that plague strains of low virulence may become prevalent under natural conditions The evidence brought forward in this respect in connexion with the supposed existence of chronic rat plague will be discussed in a later chapter, but it may be noted here that Pirie[140] referred in 1936 to a perceptible loss of virulence shown by plague strains recently isolated in the Union of South Africa *from human as well as rodent sources* Likewise Macchiavello[108] maintained that the plague cultures obtained in north east Brazil were often less virulent than those met with in other countries and were apt to undergo a total loss of virulence when exposed to adverse conditions in the laboratory Meyer et al[119] studying an instance of chronic plague meningitis in California had the impression that the recent cases of plague in the western states merely reflected the behaviour of an agent which caused mild infections with a tendency to latency

Measurement of Virulence of Strains

The following methods used in the past for measuring the virulence of plague strains though now of historical interest rather than of actual importance, may be mentioned

(1) The procedure recommended by Kolle & Martini[94] consisted of rubbing an appropriate test dose into a carefully shaved area of standard size on a guinea pig's abdomen The same procedure was also used for some time in the Haffkine Institute where however, rats instead of guinea pigs served as test animals (Sokhey[179])

(2) *Kolle & Krumbein*[93] *employed a syringe needle of standard calibre which was* dipped into a bacterial suspension of definite concentration and then introduced under the skin near the root of a rat s tail

(3) *Rowland*[1 6] *who also used rats as test animals worked with a syringe so arranged* that one turn of a screw caused delivery of 0 1 ml of the culture to be examined

(4) An alternative method adopted in the Haffkine Institute consisted of the subcutaneous administration to rats of an infective dose *containing 0 003 mg of* the spleen of a rat that had died of acute plague As was to be expected it gave grossly inconsistent results (Sokhey[179])

An exact method of determining the virulence of plague strains was introduced by Sokhey[179] who had previously devised a method of counting

the number of viable plague bacilli present in broth cultures The principle of this procedure was to make progressive dilutions each one tenth the strength of the preceding one from the growths to be tested and to spread 0 05 ml of the diluted fluids on blood agar slants which were then incubated for 48 hours Using adequate dilutions it was thus possible to obtain growth of suitably low numbers of colonies

Sokhey had also established that the strain of white mice inbred in the Haffkine Institute was highly susceptible to plague ten or even less highly virulent organisms being sufficient to cause a 100% mortality

To determine the virulence of a plague strain 0 5 ml of a broth sub culture was implanted into a tube containing 9 5 ml of nutrient broth and this second subculture was incubated at 25° 27°C for 48 hours, care being taken to keep the tube in a vertical position and free from jolts or jars

At the termination of the incubation period progressive tenth dilutions of the growth were made and used on the one hand for subcutaneous infection of mice and on the other for determination of the number of viable plague bacilli with the aid of the method mentioned above Experience had shown that in the case of highly virulent strains 0 2 ml of the 10^{-7} dilution contained the minimal lethal dose (m l d) consisting of 6 12 organisms This and the 10^{-6} and the 10^{-8} dilutions were used therefore for injecting batches of five to ten mice with doses of 0 2 ml per animal

Results were expressed as the smallest number of organisms of a given strain of *P pestis* which when administered subcutaneously killed approximately 100% of the animals within three to eleven days

The superiority of Sokhey s new test over the method of rat infection formerly used in the Haffkine Institute is shown as follows

Strain	*Old test*		*New test*		
	Number of animals infected	*Number of animals died*[a]	*Number of organisms given*	*Number of animals infected*	*Number of animals died*[a]
A	2	2 (4 2)	4	5	5 (7 8)
			40	5	5 (5 6)
B	2	2 (4 0)	8	5	1 (7 0)
			80	5	4 (10 0)

[a] The figures in brackets indicate the average number of days elapsing between infection and death

As will be noted the two strains appeared to be equally virulent when the old method was used but showed a marked difference in their virulence when tested with the aid of the procedure recommended by Sokhey

Gokhale[66] studying the oxygen uptake of three virulent and four avirulent plague strains with the aid of Warburg s manometric technique came to the conclusion that the enzymatic properties of both types were identical However as noted in chapter 2 Rockenmacher[1 1] found the catalase activity of virulent strains to be greater than that of avirulent growths and suggested that tests measuring this activity might be used

for screening the virulence of plague cultures in vitro So far no practical advantage seems to have been taken of this recommendation

Maintenance of Virulence of Strains

The problem of maintaining the virulence of plague cultures was exhaustively dealt with by Sokhey[179]

Passage through susceptible animals

Sokhey noted that the method of passage through susceptible animals, which had been almost universally used hitherto for this purpose possessed serious drawbacks While tests with highly susceptible animals gave satisfactory results, the virulence of the strains was likely to be impaired if the passage animals happened to be resistant to plague Yet no method was available to determine beforehand their receptivity to the infection Worse still the use of massive doses for the infection of the passage animals was apt to give a wrong impression of the virulence of the strains used, as is shown thus

Number of organisms per house rat (subcutaneously)	*Number of rats inoculated*	*Number of deaths*	*Number of days between inoculation and death*
64	24	0	—
64 500	25	1	17 40
132 250 000	25	25	3 4 (average)

As will be seen the strain in question though actually of very low virulence would have appeared fully satisfactory, had reliance been placed upon tests with massive inocula

Storage at low temperatures

Bearing in mind that storage at low temperatures was helpful for preserving the virulence of plague cultures Sokhey recommended replacing the method of animal passage by the following procedure

Primary cultures from human cases with severe septicaemia were obtained by plating venous blood on agar slopes After four days growth at room temperature (26°–32°C) they were tested for purity with the aid of the cultural and biochemical methods recommended by Gore (Taylor[197]) After a culture had been found pure its virulence was measured quantitatively, if found highly virulent that is if 6 to 12 plague bacilli per Haffkine Institute inbred mouse killed not less than 80% of the animals used in an average period of about seven days large numbers of subcultures on 5% rabbit blood agar slopes were made from the primary culture and the tubes were sealed on the flame and stored in a refrigerator at 4°±2°C A tube was removed from time to time to measure the virulence It was found that cultures stored in this manner retained their virulence unimpaired for at least three years

Drying from the frozen state

Still better results could be obtained by drying from the frozen state by this method virulence is maintained much longer probably indefinitely as was stated by Sokhey[19] in 1947 According to Sokhey (personal communication) the following procedure is actually used in the Haffkine Institute for freeze drying plague cultures

The strains in question are grown for 48 hours on blood agar slants at 28°C Three parts of a suspension prepared from such growths are then mixed with one part of a 20% solution of gum acacia which had been adjusted to pH 7 0–7 2 and after distribution in tubes had been sterilized for half an hour at 120°C

The mixture of the culture suspensions and the gum acacia solution is well emulsified distributed in suitable containers and mechanically shaken to ensure an even distribution of the bacilli in the menstruum After distribution in quantities of 0 1 ml in tubes of a suitable size (115 mm × 8 mm) the dispensed emulsions are rapidly frozen at -30° to -50°C The tubes are then attached to the manifolds of a cryochem apparatus and the vacuum is brought up to 200 in five minutes To avoid the possibility of thawing the tubes are kept immersed in methyl acetone at -30°C for about 30 minutes The process of desiccation is continued for 24 hours the growths are then sealed under vacuum and tested with a vacuum tester The dried growths are stored at a temperature ranging from 0° to −1°C

For regeneration a small quantity of broth (about 5 ml per tube of dried culture) is added the mass is emulsified transferred to a sterile tube and incubated at 37°C overnight The material is then planted on agar slopes

As has been shown by comparative tests gum acacia solution among all the emulsifiers tried yielded the highest survival rate (51 0%) of *P pestis* The virulence of plague cultures preserved as described above was recently found to be unabated after five years

Mitigation of Virulence of Strains

In order to evaluate the methods used for attenuating the virulence of plague strains it is necessary to determine first in what manner the transition from the virulent to the avirulent state takes place Theoretically there are two possibilities—either that all bacterial cells composing a virulent plague culture and possessing an equal degree of virulence undergo some change which renders them uniformly avirulent or that virulent and avirulent bacilli being both pre-existent in the original growth the loss of virulence is due to a process of dissociation by which the avirulent elements become preponderant or even solely present Actually there is no reason to doubt the validity of the latter assumption which as pointed out by Jawetz & Meyer[8] is strongly supported by the fact that Otten[134] was able to obtain

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avirulent subcultures by single colony picking from virulent growths of *P pestis*

Discussing the various methods for attenuating the virulence of plague strains, Jawetz & Meyer [82] aptly distinguished between (*a*) the above mentioned procedure of Otten which took advantage of a process of 'natural dissociation and (*b*) 'enforced dissociation effected by exposing the strains to adverse environmental conditions

Most important among the latter procedures were

(1) Repeated subcultivation at weekly intervals, a method used successfully by many workers more recently by Girard & Robic [63] Pirie & *Grasset,*[142, 143] *Sokhey,*[179] *Macchiavello,*[108] *and Hsue* [80]

(2) Prolonged cultivation of virulent plague strains in broth to which alcohol had been added Hetsch, [79] when introducing this method, recommended the use of gradually increased alcohol concentrations (0 5%–5%) and incubation at high temperatures (41°–43°C) Donskov & Lochov [35] claimed to have obtained avirulent variants within a month through cultivation in broth containing 10% alcohol, and within a few days when using an alcohol concentration of 15% Jawetz & Meyer [82] started with hormone broth containing 0 5% alcohol, incubating the growths for three weeks at 32°C Subcultures were then made in 3% alcohol broth and kept for three weeks at 32°C and for a further four weeks at 4°–6°C

A third procedure originally used by Burgess,[20] was to attenuate the virulence of plague strains with the aid of passage through immune animals To judge from the experiments made by Burgess himself and by Jawetz & Meyer [82] it was apparently far more difficult to obtain permanent results with this method than with those mentioned above

Russian workers (Pokrovskaya [144, 145] Korobkova [96]) were able to obtain avirulent variants of *P pestis* through bacteriophage action Otten,[134] while confirming that avirulent strains of high antigenic value could be produced with the aid of *this method found it rather laborious and time* consuming

As has been noted previously (see page 88) bubbling of air through broth cultures in the apparatus devised by Devignat led to an impairment of the virulence of the strains Devignat [31] took practical advantage of this procedure to render three virulent plague strains avirulent and also to abate a slight increase in virulence of the EV strain (initials of the child from whom it was obtained) used by him for inoculation

Describing the methods for the laboratory diagnosis of rat plague Petragnani [137] mentioned, without giving details that the virulence of plague strains could be attenuated through cultivation on bile media or media prepared with vegetable materials

TOXIN OF THE PLAGUE BACILLUS

Nature of Toxin

Petrie[138] analysing problems of plague immunity in 1929 pointed out that workers on this subject somewhat loosely applied the terms antigen endotoxin immunizing substance and vaccine almost as if they were interchangeable He himself though certain that *P pestis* possessed a specific endotoxin which was associated with the soluble protein of the bacillus and which was set free in broth cultures by disintegration of the bacilli after their death or in bacillary suspensions by extraction methods stated it as his belief that no principal difference existed between the antigenic substances present in (*a*) old autolysed broth cultures (*b*) the purer products obtained by extracting the bacillary bodies and (*c*) whole vaccines whether prepared by Haffkine s or other methods Petrie emphasized in particular that all preparations used for active immunization against plague contained toxin toxoid or a mixture of these antigens

Although a few workers such as Markl[11, 113, 114] Kossel & Overbeck[100] and Dieudonné & Otto[31] suggested that the toxin of *P pestis* might represent a mixture of metabolic products of the living bacteria and of endotoxic substances set free after their disintegration most experts are convinced that the plague bacillus has an endotoxin only as originally postulated by Rowland[156] and Besredka (quoted by Girard & Sandor[6])

However Girard and some other French workers stressed that the " endotoxin of *P pestis* differed in some ways from the typical endotoxins of other bacterial species and resembled in other respects the exotoxins Thus Girard[55] and Girard & Sandor[6] noted that the plague bacillus did not possess the glucidolipoid complex found in the endotoxins of numerous other Gram negative micro organisms On the other hand as summarized by Ramon Girard & Richou[150] the plague endotoxin was similar to the exotoxins insofar as it consisted like these of proteins could be easily transformed into toxoid and was rather thermolabile

Hence although there is no reason to revise the concept that the plague toxin is an endotoxin it will be noted that in this as in many other respects *P pestis* shows peculiar features

Preparation of Toxin

The methods used by earlier workers for obtaining plague toxin consisted either of the filtration of old broth cultures containing autolysed plague bacilli or of extraction processes The following procedures deserve mention

Filtration

Petrie[138] modifying a method devised by Markl[11, 113, 114] prepared toxic extracts thus a medium of ordinary peptone broth (pH 7 0–7 3)

with or without the addition of 1% normal horse serum, was distributed into flasks in such a manner as to ensure good access of air to the cultures which were kept at room temperature (10°–15°C) for about two months At the end of this time toluene was added to each flask and the flasks were put aside for a few days to allow the toluene to act Seitz filtration was then used

Extraction

Nucleoprotein extracts were first prepared in 1897 by Lustig & Galeotti[107] who treating suspensions of *P pestis* with 1% potassium hydroxide solution and then slightly overneutralizing with 0 5% acetic acid, obtained the nucleoprotein in the form of a white flocculent precipitate This substance conferred immunity against plague on rats if used in doses of 0 36 mg

Rowland [156] prepared toxic nucleoproteins by treating agar grown plague cultures, which had been killed with chloroform, with anhydrous sodium sulfate The extracts thus obtained were fatal to rats within 18 hours when administered in doses of 0 05–0 1 mg doses of 0 001–0 01 mg afforded substantial protection against plague infection Simple digestion of chloroform killed plague cultures in saline yielded solutions which possessed the same chemical properties as the extracts obtained with sodium sulfate and could be rendered toxic and immunogenic if care was taken to remove the chloroform which formed a loose combination with the nucleoproteins (Rowland [157])

Recently Girard [50, 54] obtained toxic extracts by three times freezing and thawing suspensions of plague bacilli according to the method of Grasset & Gory [1] then centrifuging and filtering The filtrate, if administered in doses of 0 05–0 2 ml killed mice within 6 to 36 hours

Jawetz & Meyer [8] prepared toxic extracts in the following manner plague bacilli grown at 37°C were

> suspended in buffered saline (pH 7 4) and adjusted to a density of 20 billion [20 milliard] organisms per ml These suspensions were incubated for 48 hours at 37°C and then left for 24 hours at 4°–6°C in the refrigerator The bulk of the cellular material was then centrifuged off (2 000 r p m for 90 minutes) and the supernatant fluid filtered through W Berkefeld candles and frozen in small pyrex bottles at −76°C At this temperature no deterioration of the toxic power took place over a period of at least three months

As will be mentioned later (page 125) Baker et al [6] were able to obtain from acetone dried plague bacilli, through extraction with neutral salt solutions, a water soluble and a water insoluble antigenic fraction The former was shown to contain at least three antigenic compounds including a toxic fraction (fraction II), soluble in ammonium sulfate at 0 33 saturation and at pH 7 0–7 5 and almost completely precipitated when the ammonium sulfate concentration was raised to 0 55–0 67 saturation

Baker and co-workers pointed out that it had not been possible isolate this toxin fraction in a state approaching chemical or immunolog purity all preparations being contaminated with the immunog fraction I in sufficient quantities to produce antibodies to the latte rabbits However Baker and his colleagues claimed to have succeede preparing toxins free of fraction I by serological techniques They obser that plague bacilli grown at room temperature were fully toxic but extr of such bacilli contained very little fraction I It proved possible to rem the residual fraction I by absorption with either fraction IA or IB antis The resulting absorbed extract showed no decrease in toxicity and produ antisera in rabbits capable of neutralizing plague toxin However antiserum was devoid of protective value for mice did not aggluti antigenically-complete plague bacilli to significant titre and did not r with either fraction IA or IB except to a very slight degree in sensi ring tests Its value in inducing immunity in mice and guinea pigs not as yet been investigated

Bacteriophage action

Girard[60] reported recently that he could obtain toxic lysates by adc bacteriophage to three day old cultures of his EV strain and a viru plague strain and filtering after seven hours when incomplete lysis of bacilli had taken place Mice infected intraperitoneally with 0 2 ml subcutaneously with 0 5 ml of these lysates succumbed 10–18 hours la

Girard was unable to obtain toxic filtrates from broth cultures of *P pe* incubated at 28°C before the 6th–9th day bacteriophage action seen thus greatly to accelerate the normal process of bacteriolysis

It is of interest to note in this connexion that although almost with exception pseudotuberculosis strains do not yield toxic filtrates or extra Lazarus & Nozova[101] were able to demonstrate the presence of toxic lys when subjecting 8 hour old cultures of two *P pseudotuberculosis* str of American origin to bacteriophage action Girard[60] who confirmed result ascribed the much more rapid appearance of toxic substances in lysates from these two strains than in those obtained from plague ba to the considerably quicker growth of *P pseudotuberculosis*

Plague toxoid

The transformation of the plague toxin into toxoid (anatoxin) is ea effected by adding 3 4 ‰ formalin to plague cultures and incubating 37°C for a few days As maintained by Girard[60] the toxoid thus forme not capable of protecting mice or rats against the plague toxin It is in esting to add that, according to MacConkey[109] the toxin in old autoly solutions of plague nucleoprotein had undergone a partial change i toxoid but that this comparatively atoxic substance stimulated the p duction of plague antitoxin in the horse

Heat Resistance of Toxin

The problem of heat resistance of the plague toxin is rather in As pointed out by Petrie,[138] the time factor was of marked influe well as the temperature so that storage under a comparatively lo perature for prolonged periods had the same effect as short ex (half an hour to one hour) to temperatures within the limits of 37° Petrie emphasized that according to his experience toxin soluti extracts in order to retain their toxicity, had to be kept consta temperatures not higher than 3°C

Some recent observations on the upper limits of the heat resista plague toxin showed that it became inactivated by exposure for four to 55°C (Meyer, quoted by Girard [60]), for one hour to 65°C or for l hour to 80°C (Gheltenkoff [4])

Susceptibility of Experimental Animals

Valuable data on the susceptibility of laboratory animals to endotoxin assembled by Petrie [138] may be summarized as follows

Mice

Mice are highly susceptible to the toxin, subcutaneous or intraperi administration usually killing the animals within 18 hours By mea the toxicity of broth toxins from a large number of plague strai injecting graduated doses intravenously into mice, Petrie obtaine following results

Number of toxins tested	*Average m l d ** *(ml)*
53	0 013 (1/76)
26	0 0095 (1/105)

* The average m l d of 17 broth toxins prepared from Shiga dysentery strai 0 0053 (1/190) ml

White rats

White rats usually died after 48 hours when the toxin had been subcutaneously and 24 hours after intraperitoneal administration lethal dose of broth toxins was 0 05–0 5 ml per 100 g of body w smaller amounts being needed for intraperitoneal than for subcuta administration Rowland s nucleoprotein killed the animals in do 0 1 mg

Guinea pigs and rabbits

These animals withstood large doses of the toxin a dose of 27 r plague nucleoprotein (that is a quantity 270 times the m l d for rats) failing to cause death in guinea pigs However some of the ani though not showing acute symptoms, developed a condition of mara some weeks later

Goats and horses

These animals were found susceptible to the toxin and died from toxaemia if given excessive doses

Jawetz & Meyer [6] while finding mice highly sensitive to plague toxin noted that guinea pigs could withstand at least 5 000 lethal mouse toxin doses Tame rats and ground squirrels (*Citellus beecheyi beecheyi*) combined in the experience of these workers a relatively low susceptibility to plague infection with a high resistance to the endotoxin

ANTIGENIC STRUCTURE OF THE PLAGUE BACILLUS

The antigenic structure of *P pestis* has been studied by chemical as well as serological methods Moreover some workers determined the antigenic make up of this micro organism by noting differences in the immunogenic qualities of its antigens for different rodent species According to the methods of investigation used the different findings concerning the antigenic structure of *P pestis* are here grouped under the following subheadings

Water Soluble and Water Insoluble Fractions

In the opinion of Rowland [1 6] which was shared by subsequent observers such as Morison et al [1 4] the plague bacillus appeared to be built up by two varieties of protein one soluble in distilled water or saline and containing immunizing and toxic substances the other insoluble and possessing no antigenic or toxic properties Noting that according to the observations of Rowland [1 6] the plague bacilli retained their shape after extraction of the soluble protein though appearing fragile and easily breaking up Petrie [138] drew the inference that the cell membrane was composed of the insoluble fraction

Baker et al [6] were able to obtain from buffered suspensions of a virulent plague strain grown for three days at 37°C with the aid of acetone precipitation at -70°C repeated washing with acetone and drying in vacuo a bacterial powder of high antigenicity and toxicity (see page 122) Extraction of this substance with neutral salt solutions yielded a water soluble and a water insoluble antigenic component the former being toxic and immunogenic for mice and rats but of little activity for guinea pigs and the latter while inert as far as mice and rats were concerned proving of high immunogenic value for guinea pigs when tested as an alum precipitate

Soluble fraction

As shown by saturation or precipitation with ammonium sulfate at various concentrations the water soluble fraction contained at least three

antigenic compounds, called fraction IA, IB, and II by the authors who thus described the properties of the former two substances [a]

Both fractions IA and IB gave rise in rabbits to potent antisera which apparently agglutinated all plague strains tested with the exception of one avirulent growth thought to be devoid of an envelope by Schutze [164] *but were incapable of neutralizing plague* toxin Both fractions induced immunity in mice as well as in white rats and monkeys (Meyer [118]) but not in guinea pigs

The relationship between fractions IA and IB could not be exactly defined They were almost identical serologically but fraction IB which could be obtained in crystalline form possessed a slightly lower immunogenic value Baker and his colleagues suggested that the normal antigen present in the bacterial cells might be fraction IA which contained *a carbohydrate component besides protein while the carbohydrate free fraction IB* was an artefact formed during the death and treatment of the bacilli

Again referring to this postulation in an article published in 1952 Baker et al [7] stated that

since cultures of antigenically complete plague bacilli produce viscous colonies on suitable media it may be that the surface antigen of intact plague bacilli is the viscous carbohydrate-containing Fraction IA and that crystalline Fraction IB is formed from Fraction IA during extraction through loss of the carbohydrate moiety

As shown by the serological studies of Baker et al fraction I (IA and IB) was formed by all virulent strains tested as well as by a salt stable avirulent strain whereas salt instable avirulent growths produced only traces However, incubation at 37°C seemed necessary to produce these antigens in quantity, plague bacilli grown at room temperature yielding only relatively small amounts

The observation that fraction I was practically absent in salt instable avirulent growths is of great interest in view of the claim of Bhatnagar [13] that it is the envelope substance which bestows salt stability on a suspension of the plague bacillus It is also important to note in this connexion that as established by Amies [3] through examination of wet Indian ink preparations under dark field illumination (see chapter 2 p 76) plague bacilli

which have been stripped of their envelopes begin to flocculate into small clumps to which the ink particles soon become adherent This is presumably due to the fact that the normal electric charge on the bacterial cell is removed simultaneously with the envelope In consequence the potential falls below the critical value and spontaneous agglutination is the result Removal of a surface antigen is well known to produce a decrease of suspension stability in weak electrolytes *hence the salt sensitivity* of rough variants

Electron microphotographs of avirulent and virulent *P pestis* strains are shown in fig 15 and 16

Drawing attention to the observations of other workers it should be noted that Shrivastava [175] in the course of an earlier study on the antigenic

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[a] The properties of the toxic fraction II have already been dealt with on page 1 2

ELECTRON MICRO SCOPIC PHOTOGRAPHS OF PASTEURELLA PESTIS



FIG 15
AVIRULENT P PESTIS
(×9 300)



FIG 16
VIRULENT P PESTIS
(×8 600)

structure of P pestis, had been able to isolate the following fractions from the supernatant fluid of Haffkine vaccine

(*a*) an active protein *fraction by saturating the fluid with sodium* sulfate,

(*b*) a nucleoprotein fraction by precipitation in the cold with dilute hydrochloric acid

(*c*) an active polysaccharide fraction with the aid of alcohol precipitation *in the presence of sodium acetate*

Further work in this direction was done by Seal & Mukherji[173] who were able to isolate, by precipitation with sodium sulfate *in different con* centrations, a specific soluble substance of the plague bacillus which proved highly active serologically

Continuing these investigations with the aid *of filtrates of* casein hydrolysate broth media in which virulent and avirulent plague bacilli and pseudotuberculosis bacilli respectively had been grown at 28°C for 24 days Seal[171] claimed to have isolated five different protein fractions by precipitating the filtrates at different saturations of sodium sulfate *Similar protein fractions could be isolated also from the water soluble* extracts of virulent plague bacilli grown on casein hydrolysate agar at 37°C for 72 hours Seal maintained that the antigen A obtained at one third saturation with Na SO_4 from virulent as well as from avirulent protective plague strains, was the specific antigen of *P pestis* corresponding to the envelope antigen of Schutze he stated that this fraction was practically absent in avirulent non protective plague strains and completely lacking in pseudotuberculosis strains

In a further communication[1 -] *Seal stated that*

> A polysaccharide yielding osazone resembling that of *Arabinose* with a melting *point of 166-168°C has been isolated from the supernatant of* Haffkine plague vaccine as well as from the specific soluble protein and to a less extent from the bacterial debris of both virulent and avirulent protective plague strains It is absent in non protective avirulent plague and pseudotuberculosis organisms The protective substance of the plague bacillus is *probably a* polysaccharide protein *complex*

As shortly referred to in chapter 2, Amies[3] was able to remove the *capsular substance of P pestis with the aid of* aqueous solvents such as potassium thiocyanate and thus to obtain the specific antigen of this organism in soluble form This substance, which after purification by ethanol fractionation appeared to be a simple protein was *strongly* anti genic, a few micrograms being sufficient to induce a high level of immunity in experimental animals

The question of what relationship exists between (*a*) the protein and polysaccharide antigens obtained by Seal (*b*) the immunogenic substance *isolated by Amies*[3] *and (c) the fraction* I of Baker et al,[6, 7] is of great interest and importance

Baker et al expressed the opinion that Seal s protein fractions were not sufficiently well characterized to permit comparisons Probably they were mixtures of fractions IA and IB together with other materials

According to Baker and his co workers Seal s polysaccharide possibly represented the carbohydrate moiety of fraction IA

Amies[3] maintained that his soluble surface antigen differed from the fraction IA of Baker et al insofar as it was apparently a simple protein whereas the latter was a protein-carbohydrate complex Amies added however that

" At this stage it would be unwise to attach much importance to this apparent discrepancy In view of the approximately equal potency of the two antigens it seems improbable that they differ radically in the chemical constitution of the active constituent The presence of slight impurities in one or both could account for the differences in chemical behaviour

In the opinion of Baker et al[7] the envelope protein described by Amies may be Fraction IB However further work will be necessary to establish this identity

Insoluble fraction

As recorded by Baker et al[6] in 1947 only small amounts of protein could be extracted with the aid of mild alkalis from the water insoluble residue of the bacterial powder obtained from alcohol suspensions of *P pestis* through acetone precipitation Anhydrous phenol liquified with 10–15% acetone dissolved about 25 % of the water insoluble fraction but both the phenol soluble fraction and the insoluble residue contained the antigen protecting guinea pigs In contrast to fraction I the water insoluble antigen did not produce specific protective antibodies in the serum of monkeys or man (Meyer[118])

Further discussing the water insoluble fraction in 1952 Baker et al[7] stated that this component of *P pestis* has not been studied in detail but some fraction of it is undoubtedly the somatic antigen of Schutze

" Envelope " and Somatic Antigens

Working with serological methods Schutze[163] reached the conclusion that the plague bacillus possessed two antigens one contained in its envelope and the other in its somatic portion This concept of the existence of an envelope and a somatic antigen has been accepted by practically all subsequent workers but as discussed below some of them assumed that in addition to these two a third antigenic factor was present in virulent plague bacilli Since the belief in the existence of an envelope is not shared by all observers the term envelope antigen is not very felicitous Apart from the fact however that it has been widely accepted it would be difficult to replace it To speak of a capsular antigen or following

Chertnik,[25] of a ' membrane ' antigen, seems not permissible in view of the findings of Rowland [156] recorded above

Schutze [163] stated that the 'envelope' antigen was thermolabile becoming haptenized (incapable of giving rise to antibodies) after exposure to 100°C for 15 minutes, and was destroyed after one hour at that tempera ture According to further experiments of this author [164] heating for 15 minutes at 80°C was sufficient for haptenizing the 'envelope antigen The somatic antigen was more heat stable, still provoking antibody for mation in the rabbit after one hour at 100°C and reacting in vitro even after three hours' exposure to the same temperature

The conclusions reached by Schutze [163] when comparing the antigenic structure of the plague and pseudotuberculosis bacillus may be summarized as follows

Species	*Antigen*		
P pseudotuberculosis	Flagellar	Smooth somatic	Common rough somatic
P pestis	Envelope	—	

Bhatnagar [13] postulated that the pseudotuberculosis bacillus possessed both group and type specific somatic antigens

Studying the antigenic structure of the plague bacillus with the aid of chemical procedures Kurauchi & Homma [101] were in agreement with Schutze that this micro organism possessed two independent antigens—a somatic antigen which shared immunogenic characteristics with the pseudotuberculosis bacillus, and a capsular antigen Considering that the latter alone was of importance in conferring immunity against plague infection they called it the specific immunizing fraction '

The two workers extracted this substance by a procedure not specified in their report, purified it by the ' acid acetone method and concentrated it They found it contained 1 84% nitrogen, 8% ash, and 40% reducing substances

Experimenting with this substance, Kurauchi & Homma found it to confer a stronger immunity when administered twice to guinea pigs and rats than ordinary plague vaccine Three monkeys which received two injections also withstood a challenge infection with virulent plague bacilli but a fourth, which had received one dose only, succumbed It was found however that after absorption of the specific soluble substance by metallic salts satisfactory results could be obtained with a single injection

Serological Properties and Virulence

Schutze,[16] as well as Wats et al [-0] and Jawetz & Meyer,[8] [83] emphasized that it was impossible to distinguish with the aid of serological tests between virulent and avirulent smooth or rough plague bacilli Jawetz & Meyer [81] also stated that a factor which enhanced spreading and capillary per

meability had been found in extracts of both virulent and avirulent *P pestis*

Russian observers (Fadeeva [36] Gheltenkoff & Khvorostukina [47] Korobkova [99]) postulated that virulent plague bacilli possessed an antigen similar to the Vi antigen in the salmonellae but Jawetz & Meyer [8] were unable to confirm this claim Before dealing with the explanation they gave for the antigenic difference between virulent and avirulent plague strains it is necessary to set forth the opinion held by Otten regarding the antigenic structure of *P pestis*

Otten [131] comparing the immunizing power of his avirulent Tjiwidej strain with the potency of other avirulent plague strains came to the conclusion that

" most of these strains possess an antigen especially active in guinea pigs but producing less or even slight effect in rats The strain Tjiwidej on the other hand is the best antigen for rats but must be put behind others as regards guinea pigs This difference is of a qualitative nature as it cannot be overcome by increasing the dose

In a subsequent paper [131] Otten maintained that this difference remained manifest when tests were made with heat stable (somatic) antigens of these various strains after the heat labile (envelope) antigens had been rendered inactive through heating Otten postulated therefore that the somatic antigen was composed of a guinea pig antigen and a rat antigen present in different proportions in the various strains

Jawetz & Meyer [8] while considering Otten s hypothesis as insufficient were also disinclined to believe that different immunity mechanisms due to variations in the resistance to plague toxin might be at work They assumed that some antigenic constituent chemical group or property, present in most live vaccines but often absent in killed ones might explain the divergent results obtained in different animal species Mice might not be in need of this factor thus becoming easily immunized with killed vaccines It was however essential for guinea pigs which therefore could be protected by specially prepared killed vaccines only—for instance the sugar vaccine of Minervin et al [1] and Haffkine vaccine (Sokhey [180] Sokhey & Maurice [190])

Generally speaking Jawetz & Meyer assumed that there was

" little doubt of the presence of some property or chemical group responsible for virulence in the plague bacillus since some natural dissociants seem to be identical with virulent organisms in all respects except for a complete loss of virulence

They stated in a later paper [83] that the agent at work might be of an enzymic nature Rockenmacher s [154] claim that the catalase activity of virulent strains is greater than that of avirulent growths is of great interest in this connexion

It deserves attention however that in the course of their recent work Baker et al [7] found no evidence for the existence of a definite entity responsible for virulence as Jawetz & Meyer suggested

NATURAL RESISTANCE TO PLAGUE

Although as will be discussed later most animals other than rodents are insusceptible to plague, the immunological aspects of their resistance seem to have been fully studied only in the case of birds

Birds

The natural resistance of birds to plague cannot be considered as absolute Thus Giaxa & Gosio (quoted by Dieudonne & Otto[31]) found starving pigeons and sparrows susceptible and as observed by Albrecht & Ghon[2] and others intravenous or intraperitoneal administration of large doses of highly virulent plague bacilli to normal birds occasionally produced fatal infections However, these exceptions do not invalidate the rule that under ordinary circumstances birds are resistant to plague

London[106] ascribed this resistance to the presence of specific humoral antibodies in normal birds, but both Flu[39] and Hoessli (quoted by Meyer[119]) failed to confirm this claim As maintained by Meyer,[118] no credence should be given to the assumption that the insusceptibility of chickens or other birds was due to their high body temperature (42°C or 107 6°F) Thus, in the opinion of this author, ' phagocytic destruction of plague bacilli by fixed macrophages in all probability constitutes an important if not the determining factor in the natural resistance of birds to plague infection

It is interesting to note that this insusceptibility to plague is not shared by chick embryos (Buddingh & Womack,[19] Jawetz & Meyer[83]) Jawetz & Meyer observed in this connexion that, while virulent plague bacilli intro duced in small numbers multiplied rapidly and caused the death of chick embryos within four to eight days small numbers of avirulent bacilli failed to proliferate freely Another interesting point was that a potent plague serum was unable to protect the embryos against *P pestis* because, as these workers assumed, a cellular defence mechanism, without which the serum could not exert its protective action, was still absent

Rodents

Although plague is primarily a disease of the rodents even these animals are by no means uniformly susceptible to the infection Not a few of the numerous species composing the order *Rodentia* have been found to be rather, or even completely resistant to experimental infection with *P pestis* More important still, marked differences in plague susceptibility, due either to the existence of differently receptive races or to seasonal influences, have been found even in the species most concerned in the perpetuation of the infection

While the mechanism underlying this racial or seasonal resistance seems not yet to have been fully elucidated all available evidence tends to show that as in the case of the birds so also in naturally plague resistant rodents a principal role is played by cellular defence factors

Proof for this contention seems to have been furnished by Bhatnagar & Shrivastava[11] who worked with Bombay rats (*Rattus rattus*) so resistant to the infection that 91% of the animals survived subcutaneous injection with $3\text{-}4 \times 10^3$ virulent plague bacilli Through a continued study of the blood picture of the infected animals the two observers were able to show that during the first 48 hours after infection the polymorphonuclear leuco cytes reacted against the invaders Then an increasing number of clasmato cytes appeared in the blood and obviously continued the fight until on the seventh day simultaneously with the disappearance of these cells and the appearance of young polymorphonuclears the condition of the animals improved

Similarly Meyer[118] found that in the comparatively resistant cotton rat (*Sigmodon hispidus hispidus*) the reticulo endothelial cells of the liver were instrumental in removing *P pestis* It is also noteworthy that as shown by Larson (quoted by Meyer[118]) the fatal termination of plague infection in monkeys was preceded by a drop in lymphocytes and monocytes

An interesting observation on the seasonal changes in plague suscepti bility was that according to Meyer[118] guinea pigs surviving from litters other members of which had been fully amenable to infection during the summer became quite resistant during the winter Washed peritoneal exudate cells of such animals were able to destroy in vitro several hundred plague bacilli within 3-24 hours

Plague in hibernating wild rodents will be dealt with in a later study but it should be mentioned here that according to Gaiski,[43] who devoted much effort to a study of this problem the resistance of such animals during hibernation was due not to immunological factors but to bacteriophage action

Man

No convincing evidence is available to show that a natural immunity to insect borne plague exists in man In particular there is no secure foun dation for the belief that certain races are less susceptible to this type of the infection than others the differences observed in this respect being in all probability due to greater or lessened chances for infection and not to racial differences in susceptibility The existence of so called healthy carriers of bubonic plague postulated by a few observers must not necessarily invalidate the rule that no natural resistance to insect borne plague exists in man because such individuals having probably passed through an unnoticed attack of pestis minor were presumably convalescent and not healthy carriers

It cannot be claimed however, that the same explanation holds in the case of the carriers of pneumonic plague observed upon se occasions or more generally speaking of persons who did not cor this highly infectious form of the disease in spite of taking no precau when in prolonged close contact with pneumonic plague patients instance, in wards (Wu Lien teh[201]) While it is possible, therefore instances of a resistance to pneumonic plague infection exist they a such rare occurrence as to be of no practical importance

MECHANISM OF ACTIVE IMMUNITY TO PLAGUE

Earlier views on the mechanism of active immunity to plague may be summarized

Infecting white rats by the intraperitoneal route, Markl[115] *found* lysis and phagocytosis of the bacilli competed with the rate of multiplica of those remaining free in the exudate

Similar results were obtained by Rowland[158] with intraperitone and subcutaneously infected rats Finding that virulent bacilli multip slowly in the body of vaccinated rats and that avirulent bacilli did like even in normal rats while virulent infection progressed rapidly in the la he reached the conclusion that the essential factor in plague immu *was that which affected the multiplication of the bacillus*

Commenting upon these findings of Markl and Rowland Petri stated that the part taken by phagocytosis in the defence mechan could be explained on the basis of an opsonic action He noted in connexion that Douglas (quoted by Brooks[18]) had found a good opso response in man after inoculation with Haffkine vaccine while Brook had the same result when injecting white rats with the soluble protein *P pestis* However the bacteriolytic part of the defence mechanism co *not be easily explained, since most workers failed to demonstrate* a ba ricidal element in immune serum

Malone et al,[111] estimating the bactericidal power of rat blood a afterwards infecting these animals with a standard dose of plague baci found that the results obtained by these two methods did not tally a ficially immunized rats having a better chance for surviving after challenge infection than the moderately resistant Bombay rats or the ful susceptible Madras rats, regardless of their haemobactericidal pow *Malone and his co workers assumed* therefore either that the blood immune animals possessed in vivo some antibacterial quality which cou not be demonstrated in the laboratory, or that part of their immunity w derived from sources outside the blood stream (tissue or antiblastic immunity)

Role of Phagocytosis

Dealing with the behaviour of *P pestis* in normal and immune experimental animals (guinea pigs and mice) Jawetz & Meyer[8] found that if smaller inocula were given to immune animals the bacilli were frequently fixed at the site of infection and slowly destroyed there Administration of large numbers of virulent bacilli led to their rapid distribution in actively immunized as well as in normal animals but in the case of the former this was followed in a few days by the disappearance of the micro organisms first from the blood then from the liver and spleen and afterwards from the lymph nodes and site of infection

Continuing their studies Jawetz & Meyer[81] were able to confirm the above mentioned opinion of Petrie as well as the results of recent work by Joukov Verejnikov & Fadeeva[88] by showing that the serum of animals immune to plague was unable to destroy or lyse *P pestis* either in vitro or in vivo in the absence of phagocytic cells Whole blood of plague immune animals could destroy a much greater number of plague bacilli than blood of normal animals this activity being found to be primarily inherent in the plasma which probably either made the bacilli more easily digestible for the phagocytes or conferred specific qualities upon the latter

Assessing their results as a whole Jawetz & Meyer[81] found that the problem of plague immunity was rather complex and that in all probability different mechanisms may be responsible for a greater or lesser share in the total picture of immunity in the various laboratory animals

In a recent comprehensive survey of the problems of plague immunity Meyer[118] pointed out that as shown by the investigations of Burroughs[1] the intact skin was capable of barring the entrance of plague bacilli to a considerable degree Since however infection through flea bites led to a direct invasion of the capillaries a further defence mechanism furnished by the leucocytes and auxiliary elements had to come into play

In order to study the factors involved Meyer and his co workers made ample use of cytograms a method originally devised by Rowland[158] and also used by Pokrovskaya & Kaganova[116] to whose work reference is made below Experimenting with different animals particularly with monkeys and mice Meyer and colleagues used the following technique

" An area of the ear or sternum is inoculated with plague bacilli and a fine needle is inserted into the area Samples of the tissue fluid are aspirated at regular intervals and suitably stained smears are made for cytological examinations

As stated by Meyer during the first two hours after infection when in normal as well as in moderately immune animals the injected area became markedly oedematous the films prepared from either normal or immune animals did not differ strikingly Then however marked differences began to appear, as shown by the following summary of Meyer s principal findings

(1) Between two and eight hours after injection the plague bacilli became agglutinated in immune but not in normal animals Many single organisms were swollen and indis

tinct in the immune animals and in contrast to the non immune the number of the bacilli was definitely reduced

(2) With the arrival of polymorphonuclear leucocytes between four and six hours after phagocytosis became pronounced in the immune animals while only occasional cells of the non immune ingested a few bacilli By the 12th hour phagocytosis dominated the microscopic picture in the immune animals while in the non immune animals not more than 10% of the leucocytes were able to ingest plague bacilli in the early stages of the infection Thus while in the exudate of the immune host phagocytosis competed successfully with the rate of multiplication of the plague bacilli in the non immune animals the bacilli multiplied extracellularly without hindrance and phagocytosis practically ceased by the 24th hour

(3) Within 24–36 hours the serous tissue fluid in the immune animals became converted into a dense cellular exudate Simultaneously the leucocytes clumped and the exudate condensed thus aiding fixation of the bacilli The few micro organisms which did not become phagocytized were ultimately taken up by polyblasts of the macrophage series

Summing up the problem of plague immunity in general Meyer considered that

phagocytosis is the most important mechanism which animals and man use in guarding against and disposing of a plague infection The mesenchymal tissue cells are responsible not only for cellular immunity but for humoral defense as well Their cytoplasm modified by effective contact with antigen develops adaptive enzymes and thus circulating antibodies which in turn remove the antiphagocytic property of the slimy envelope of the plague bacillus The effectiveness of the mechanism and consequently the fate of the host are determined by the balance between bacterial multiplication and the efficacy of the clearing mechanism

"Organ" and Local Immunity

Some evidence exists that under certain circumstances even in immune animals the lungs are less resistant to plague infection than organs like the liver and spleen

It should be noted in this connexion that, as established by Batzaroff[11] and confirmed by other workers such as Girard[58] and Jawetz & Meyer,[8] animals partly immune to plague and therefore succumbing to a challenge infection after prolonged illness regularly showed marked lung involvement in the absence of liver and spleen lesions

Discussing these findings, Jawetz & Meyer[80] drew attention to the observation of Sprunt & Camalier[19] that the resistance of the lung to bacterial infections was lowered through circulating toxins Experimental animals like guinea pigs, monkeys and also mice if partially immune could withstand the original impact of the infection but at the same time plague bacilli persisted and slowly multiplied at the site of the infection and in the regional lymph nodes, and disintegration of part of the micro organisms led to the liberation and circulation of endotoxins The latter led to the establishment of a locus minoris resistentiae (site of least resistance) in the lungs so that pneumonic foci could become established even as

the result of a minor bacterial invasion That no foci of the infection developed in the liver and spleen was probably due to an enhanced cellular activity which not only prevented multiplication of the plague bacilli but also resulted in an increasing toxin resistance

The explanation given by Pokrovskaya & Kaganova [146] for the low resistance of the lungs to plague infection was that the cells of this organ were lacking in phagocytic power Discussing the pathogenesis of two human cases of chronic meningitis and encephalitis observed in California Jawetz & Meyer [8] similarly assumed that the bacilli reaching the brain early in the disease could persist and slowly multiply there because they were protected from phagocytic action

Petragnani [157] claimed that a local immunity could be created through instillation of avirulent plague bacilli into one eye of guinea pigs Such animals survived if some weeks later virulent bacilli were instilled into the same eye but succumbed to generalized plague if the unprotected eye were infected

Anti infectious and Antitoxic Immunity

A distinction between an anti infectious and an antitoxic immunity was made by some observers

In the experience of Jawetz & Meyer [81] a high antitoxic resistance did not by itself protect against plague infection whilst a high anti infectious immunity was invariably accompanied by a certain degree of toxin resistance This was in agreement with the results previously obtained by Markl [115] but not with those of Rowland [158] who claimed that anti infectious immunity was conferred by the administration of plague toxin or toxoid

In the opinion of Otten antitoxic immunity could be more easily produced than anti infectious immunity but was of little value under natural conditions where the invasive power of the plague bacilli was the decisive factor Jawetz & Meyer [81] while admitting that in anti infectious immunity the defence was primarily directed against bacterial multiplication and not against liberated endotoxin considered it as likely

" that active or passive antitoxic immunity can temporarily ward off the effects of products of bacterial disintegration and thereby permit the development of an efficient defense mechanism directed against the multiplying bacilli

As established by these workers [8] fairly good antitoxic immunity could be produced in mice by either live avirulent or formalin killed vaccines provided a restimulating injection was administered in addition to the initial dose

ACTIVE IMMUNITY TO PLAGUE

It is certain that human beings as well as rodents if surviving a plague attack are apt to be resistant to the infection However owing to the fact that until recently instances of recovery from severe forms of the

be properly assessed Almost invariably it was impossible to arrange for actual tests in the field on a sufficiently large scale and moreover most difficult adequately to interpret the effects of these methods in man It would seem at first glance that tests made in the laboratory ought to have given less doubtful results but no exact methods were available for this purpose and great uncertainty existed regarding the proper choice of test animals

Discussing the latter problem Taylor[19] considered both the lower monkey species used in plague work and the rabbit as unsuitable for testing vaccines on account of the variability of their response to challenge doses

Guinea pigs also appeared unsuitable for vaccine work both on account of their resistance to toxins and because they did not respond well to immunization

Trapped wild rats (*R rattus*) which were largely used in the Haffkine Institute were by no means ideal In this connexion Taylor pointed out the necessity of transporting susceptible rats from Madras to Bombay which resulted in a considerable mortality Moreover the animals proved very susceptible to the toxic action of the vaccine frequently 20% to 30% succumbing when the usual test dose of 0 5 ml was administered The average immunity of the rats on the other hand was only in the vicinity of 30% to 40% Therefore wild rats though useful for tests on broad lines were unsuitable for biological standardization or the elucidation of finer differences between the vaccines tested

Laboratory bred albino rats were found less susceptible than the wild *R rattus* and irregular in their response to infection with various doses

However as announced by Taylor Sokhey had recently established that the strain of white mice inbred in the Haffkine Institute had proved excellent These animals were as a rule fully protected when receiving 0 03 ml of Haffkine vaccine while 10 times this amount was required to produce toxin deaths At the same time their susceptibility to the challenge infection was very regular

The great value of the white mouse for testing plague vaccines has been recently endorsed by Meyer et al [11] who reached the conclusions that

(1) the lyophilic envelope fraction IB antigen is probably essential in the maximum protection of man

(2) since this antigen is equally indispensable in the protection of mice against plague it is deemed advisable to use mice rather than guinea pigs to measure the immunogenic potency of a plague vaccine

The method of biological standardization of plague vaccines introduced by Sokhey to replace the former unsatisfactory procedures was based upon the use of the method for measuring the virulence described earlier (page 116) it consisted in determining the minimum quantity of a plague vaccine that would save a white mouse challenged seven days after administration of

the second dose of the vaccine under test As described by Sokhey & Maurice[189] the procedure was as follows

The quantity of the vaccine to be administered was divided in two equal doses and given subcutaneously with a seven day interval Five mice were used for each quantity of vaccine and four to five quantities were used at a time The minimum quantity that saved at least three out of five mice during a 25 day period of observation commencing with the day on which they had been given the challenge dose was taken as the protective dose of that particular vaccine The results for all the vaccine quantities used had to be consistent among themselves and the result of duplicate tests had to tally The mortality of the control animals had to be 100/

Again discussing the method of biological standardization, Sokhey & Maurice[190] stated that they had obtained good results with Haffkine vaccine in guinea pigs as well as in white mice, as shown thus

Number of animals	*Total vaccine dose (ml)* [a]	*Challenge dose (number of organisms)*	*Number of deaths*
5	0 80	1 million	1
5	1 25	1 million	0
5 (controls)	—	1 million	5

[a] Given in two equal doses at seven day intervals

It should be noted that satisfactory results with guinea pigs have been recently reported also by Wayson et al [03] and by Meyer[117] who worked with vaccines treated with alum or other synergists

Comparing the efficacy of killed vaccines for white mice (body weight 25–30 g) and white rats (200–270 g) Sokhey & Habbu[185] found that, contrary to the statements made by some other observers the latter animals could be equally well protected by Haffkine broth vaccine grown at 28°C and agar vaccine prepared from cultures incubated at 37°C

Vaccine	*Mouse protective dose (ml)*	*Rat protective dose (ml)*
Haffkine vaccine (28° C) four week s growth	0 004	0 030
Agar vaccine (37° C) 1 000 million organisms per ml	0 004	0 028

Relation of virulence to potency

Summarizing the literature available up to 1936 Pollitzer[147] state that the necessity of using the most virulent strains possible for the prepara tion of plague vaccines had been stressed by pioneers in this field such a Haffkine and the German Plague Commission as well as by all subsequei workers However, some recent observations have thrown doubt upon th absolute validity of this claim Thus Schutze,[165] experimenting wit Haffkine type vaccines prepared from virulent and avirulent plague strain came to the conclusion that " virulent cultures do not result in more poter vaccines for either rats or mice than do avirulent ones Likewise Sokhe & Habbu[185] obtained the following results when comparing two heat

killed vaccines (A and B) prepared from strains showing marked differences in virulence

Vaccine	Minimum lethal dose (number of organisms)	Minimum protective dose (number of organisms)
A	5	6 500 000
B	300 000 000	5 300 000

Sokhey & Habbu concluded that these experiments had not been carried to a logical end but the observations so far made showed that even considerable differences in the virulence of strains did not make any difference to the protective power of a heat killed vaccine

Assessing the comparative value of virulent and avirulent plague strains for the manufacture of killed vaccines it must be kept in mind however that while the antigenic value of the former is and remains equal under proper conditions of storage avirulent growths show great variations in potency (Sokhey [180]) and as will be discussed later (page 153) even the antigenic value of one and the same strain may deteriorate

Relation of toxicity to potency

The relation of the toxicity to the potency of the vaccines was thus summarized by Pollitzer [117] in 1936

Haffkine was convinced that a definite degree of toxic reaction was desirable and necessary for effective immunization in fact the dosage of his prophylactic fluid for man was originally determined on the basis of toxicity Rowland though pointing out that the toxicity of a vaccine could be abated without interfering with the antigenic properties was in accord with Haffkine s views Taylor summarizing the subsequent experiences of the Haffkine Institute admitted some influence of storage on the toxicity as against the potency but also insisted upon a close relationship between these two properties not only in the case of Haffkine s fluid but in that of all effective plague vaccines Petrie discussing the theoretical aspects of this problem stated that there was no clear proof of the existence of an independent antigen apart from the toxin and the opsonic antigen which conferred an active immunity against the living culture The rapid induction of immunity conferred by the nucleoprotein seemed in Petrie s opinion to bear a relation to the rapid absorption of plague toxin or the rapid opsonic response rather than to the slower action of a bacterial antigen of the thermostable type

Considering this evidence Pollitzer stressed the fact that as shown by some of the above mentioned and also by other observers the toxicity of plague vaccines could be abated without any considerable loss in potency Thus as shown by Schutze [163] and confirmed by Sokhey & Maurice [183] incubation of broth vaccines at 37°C instead of 27°C reduced their toxicity without interfering with their antigenic properties

Measuring the toxicity of broth and agar vaccines by determining the doses which killed 50% of the white mice used (body weight 26–27 g) Sokhey & Habbu [18] obtained the following results

Vaccine	Toxic doses (ml)
Haffkine vaccine (28 C) four weeks growth	0 2
Agar vaccine (37 C) 1 000 million organisms per ml	1 0

Sokhey & Habbu concluded from this and confirmatory tests that vaccine was much less toxic than broth vaccine At the same time, how they recorded encouraging results when using formalin instead of to sterilize the vaccine obtained from four week growths in casein hy lysate

Method of sterilization	*Toxic dose for mice (LD)*
Heat (54°C) + phenol (0 5%)	0 2
Formalin (0 05%) + phenylmercuric nitrate (1 mg per 100 ml)	0 4
Formalin (0 075%) + phenylmercuric nitrate (1 mg per 100 ml)	0 5

Since, as will be discussed later (see page 144) the new casein hydroly vaccine was an excellent prophylactic, these observations support the v that it is possible to reduce the toxicity of plague vaccines with biasing their antigenic value

Comparative potency of supernatant fluid and solid portion of vaccines

While some early observers such as the German Plague Commissio were of the opinion that the antigenic potency of Haffkine vaccine vested in the solid portion containing the body substances of the bac most workers were agreed that since the antigenic fraction of *P pe* responsible for the production of immunity apparently passes into soluti the whole value of this vaccine resides in the fluid portion (Taylor[1]

Dealing again with this problem recently, Sokhey & Habbu [183] confirm that, in the case of broth vaccines, the total protective power resided the supernatant fluid, regardless of whether incubation at 27°C or 37 had been used They likewise observed that when 48 or 72 hour a growths incubated at 37°C were suspended in water and the bacilli w left in this for three to seven days the total immunogenic power v contained in the clear fluid separated from the solid part of the suspensi However, in the case of growths incubated at 27°C the clear fluid obtain in the same manner had very poor protective power

Sokhey & Habbu suggested that it might be advantageous to use o the supernatant fluid of broth vaccines grown at 27°C or of suspensio of agar cultures incubated at 37°C for inoculation because such vaccin would be less toxic

Methods of killing vaccines

Heat The pioneers of antiplague inoculation resorted to heat to rend their vaccines innocuous and this practice has been followed until recent by most workers

Haffkine first sterilized his broth vaccine brews by exposing them f one hour to 70°C but soon reduced the temperature to 50°–55°C and t

period of heating to 15 minutes While this period remained the standard in the Haffkine Institute temperatures of 60°–64°C were used for the pre paration of broth vaccine after the departure of Haffkine in 1905 and until Sokhey demonstrated that they exerted a most deleterious effect on the potency of the brews The method of applying a temperature of 55°C for 15 minutes (Taylor [19] Sokhey [1 9]) was therefore once more adopted

Antiseptics While sterilization by heat remained until recently the standard method for the preparation of Haffkine vaccine other workers began to use antiseptics for the same purpose Thus mere phenolization at room temperature was employed for the manufacture of the agar grown Lister Institute vaccine and was also utilized by Burgess [0] Row and Nikanorov (quoted by Pollitzer [11]) worked with glycerol but their products, which were destined for the treatment of plague patients rather than for prophylaxis had the drawback of requiring prolonged storage before use

Formalin which had previously been used by Batchelder [10] to prepare plague antigens for laboratory purposes was actually used for the manu facture of plague vaccines by French workers as well as in the USA (Meyer [11]) during the second World War and has also been adopted for the production of the new casein hydrolysate vaccine in the Haffkine Institute (Sokhey & Habbu [186])

Comparing different methods of killing vaccines Sokhey & Habbu [184] recorded the following results

M th I	*M p t tl e d) aga a t e*	*b th c ct e*
Heating at 54 C	0 0065	0 006
Merthiolate (1/500 000)	0 0068	0 006
Sulfathiazole (1)	0 0070	–
Alcohol (Felix)	0 0160	–

Types of killed vaccines

(1) *Haffkine s vaccine* Bearing in mind that owing to the introduction of the new casein hydrolysate vaccine the methods of manufacturing the classical Haffkine fluid have become a matter of historical rather than of actual interest it is proposed to deal here only with those principles involved in its preparation which remain important for future work

(*a*) *Incubation temperature* The incubation temperature of about 27°C originally recommended by Haffkine has been found best for the pre paration of fluid plague vaccines by most subsequent workers As confirmed by the exact tests of Sokhey [178] broth vaccines grown at about 27°C were more potent than those incubated at 37°C because growth of *P pestis* was maximal at or near the former temperature Further as stated by Taylor [197] incubation at 27°C was also beneficial insofar as it restrained the develop ment of many other micro organisms including the pasteurellae tested

It was likewise a convenience that during the greater part of th average room temperature at Bombay was about 27°C

The lesser toxicity of broth vaccines grown at 37°C noted a point deserving great attention but as has been found by Habbu,[185] the use of formalin instead of heat for manufactur vaccines seems to go a long way towards restoring a balance in incubation at 27–28°C

(b) *Period of incubation* Though Haffkine had rec incubating his vaccine for six weeks, this practice was not strict to by his successors and finally an incubation period of four made the standard

(c) *Storage* It is generally agreed that storage of the vaccine, while reducing its toxicity (Stevenson & Kapadia [195]) did an unfavourable influence on its potency Taylor [197] noted in this that after a slight drop in potency which occurred during the fi the vaccine even if stored at room temperature, retained a high in value for a year and lost but little of its potency during the fol months Sokhey & Habbu [18] even found that a batch of th which had been lying at room temperature for about 10 years st a very high protective power They also made the following ob comparing the potency of two lots of broth vaccines, grown for f at 28°C and heat killed, with that of two lots of heat killed agar grown at 37°C and stored at different temperatures

Media	Storage temperature (C)	Storage period (weeks)	Potency (mouse protective (ml)
Broth	0		0 0052
	45	8	0 0040
Agar	0		0 0035
	45	6	0 0060

It will be noted that, in contrast to agar vaccine broth vaccine even if stored at 45°C

(2) *Casein hydrolysate direct vaccine* The preparation and p of the new casein hydrolysate plague vaccine were thus described b & Habbu [186] (see also Sokhey, Habbu & Bharucha [187])

A very much improved plague vaccine with a consistently high p value can be prepared by the use of Mueller & Johnson s casein hy The medium is so prepared that controlled digestion produce which is entirely free of proteins and is perfectly limpid with a lig tinge It is adjusted to contain 270 mg of nitrogen per 100 ml

The medium is distributed in quantities of 1 litre in modified flasks of three litre capacity and is seeded with specially selecte

of *P pestis* preserved by drying from the frozen state The growths are incubated at 28°C for two weeks Killing is done by the addition of 0 1% formalin and phenylmercuric nitrate is used as the preservative

The mouse protective dose of this vaccine is 0 004 ml It is much less toxic than the previous vaccine since 0 6 ml as opposed to 0 2 ml is needed to kill a mouse The keeping qualities of the new vaccine are very good stored at 37 C for 18 months it does not show any loss of protective power

(3) *Agar grown vaccines* The technique originally adopted by the German Plague Commission [41] for the manufacture of agar grown plague vaccine consisted in washing off the growth of two day old virulent cultures with broth or saline heating the collected washings for one to two hours at 65°C and afterwards adding carbolic acid so as to obtain a concentration of 0 5%

The modifications of this procedure introduced by subsequent workers may be summarized thus

(*a*) *Media* While most of the earlier workers did not specify the composition of the solid media they used Burgess [0] recommended a whole meat agar as being simple to prepare and yielding abundant growth Special media preferred by recent workers were glycerol agar (Barreto [9]) hormone or hormone sulfite agar used for the preparation of the US Army vaccine during the second World War and blood tryptose beef heart agar used by Wayson et al [03] for obtaining vaccines for experimental purposes

(*b*) *Temperature and period of incubation* The earlier workers do not seem to have been particular as to the temperature of incubation apparently using temperatures ranging from 27°C to 30°C Schutze [163] seems to have been the first who insisted on incubation at 37°C because he assumed that in this way growths rich in envelope antigen resulted Sokhey [178] also found that agar vaccines grown at 37°C were far more potent than those obtained at 27°C when the mouse protective dose was 0 1 ml as against 0 002–0 004 ml in the case of the former However, he did not ascribe this difference to the presence or absence of an envelope but pointed out that the plague bacilli grown at 37°C were bigger and that consequently the actual bulk of agar vaccine obtained at this temperature was greater than that of 27°C vaccine containing the same number of organisms Sokhey assumed however that this was probably not the only factor involved

An incubation temperature of 37°C was also found preferable by other recent workers such as Grasset [68] The US Army vaccine seems to have been grown at 30°C but Wayson et al [03] incubated the vaccines they prepared for comparison with it at 39°C

The periods of incubation adopted by the various workers *ranged from* 40 to 72 hours

(c) *Standardization* Besides the usual methods of standardization (actual counting or opacity tests) some special procedures were utilized Burgess,[70] *who formerly adjusted his plague vaccine to contain 3,000 million* bacilli per ml (the standard previously adopted for the Lister Institute *vaccine) afterwards used a standard of 1 5 mg of dried bacterial substance* per ml and considered both these strengths as equivalent

A more expedient method originally devised by Japanese workers was to weigh the sediment obtained by centrifuging the vaccine and then to *dilute the latter so that each ml contained 6 mg of the sediment (Pollitzer* [147])

Sokhey [181] used agar vaccines adjusted to contain 1,500 million plague bacilli per ml so as to give them the potency possessed by broth vaccine incubated at 27°C for four weeks A lower standard (1,000 million) was adopted by Barreto [9] a higher for the Army *vaccine (2,000 million per ml)*

Comparing agar- and broth grown plague vaccines it has to be noted *that the former can be manufactured more easily and expeditiously than the* latter This difference is of great importance in emergencies when it will *be possible quickly to prepare agar grown vaccines from freshly isolated* local strains In areas where plague occurs perennially however, stocks of *broth grown vaccine, which in contrast to agar grown vaccine possesses* excellent keeping qualities can be prepared beforehand

Sokhey & Habbu,[182] *while considering the protective power of Haffkine* vaccine grown for four weeks at 28°C roughly to equal that of agar vaccines *obtained through cultivation at 37°C, admitted that the former was more* toxic It was on account of this drawback that new research work was *started which led to the introduction of the casein hydrolysate vaccine* As has been noted, this combines high potency with low toxicity

(4) *Pseudotuberculosis vaccine* As a result of previous work by Rowland and others (see Pollitzer [147]), a vaccine prepared from formalin killed pseudotuberculosis bacilli (containing 4,000 million per ml) was introduced for human antiplague inoculation in Madagascar (Boye [16, 17]) According to Boye this product protected about 51 % of domestic mice against infection with a tenfold m l d of plague bacilli

(5) *Lipo vaccine* A lipo vaccine, also introduced by French workers was prepared according to Boye,[16, 1] in the following manner

Plague bacilli were grown for 36 hours at a temperature of 34°C on agar of pH 7 4 then washed off with saline containing 5 ml of formalin per litre After storage at 37°C for ten days 12 5 g of centrifuged and dried bacilli were suspended by a special process in an oily excipient

Pons & Advier,[149] challenging grey mice with 200 virulent *P pestis* ten days after prophylactic inoculation, found the lipo vaccine to give a 70%

survival as compared with 51 5% in animals protected with aqueous vaccine and 20% in the controls Almost equally good results were obtained when both plague and pseudotuberculosis bacilli (6 000 million of each species per ml) were used for preparation of the lipo vaccine

(6) *Sugar vaccine* As summarized by Pollitzer[117] Minervin et al[1] *obtained remarkably good results when protecting sisels with heat killed* suspensions of avirulent plague bacilli in a saccharose solution Korobkova et al[89] worked with a similar vaccine which they called AD vaccine (vaccin adenature) However in a subsequent report Korobkova[88] recommended using virulent instead of avirulent plague bacilli for the preparation of this vaccine and also stressed the necessity of sterilizing it without the application of heat so as not to damage the heat labile Vi antigen of *P pestis* supposed by her to exist Consequently she added to a concentrated suspension of virulent plague bacilli grown at 37°C a double volume of 80% saccharose solution and let the mixture stand at room temperature for 20–25 days to effect sterilization

Korobkova found that two injections of this vaccine protected 82–100% of white mice against a challenge infection with highly virulent plague bacilli and that protection was also afforded to 50–85% of guinea pigs which had been given three vaccine doses A sugar vaccine prepared with the avirulent EV strain was less potent The addition of 0 05% agar to the sugar vaccines increased their protective power

(7) *Precipitated vaccines* The procedures adopted by Wayson et al[203] for the preparation of plague vaccines with the aid of precipitation methods were as follows

(*a*) *Alcohol precipitated vaccine* Two volumes of 95% ethanol were added to the phenolized saline suspensions of virulent plague bacilli grown on blood tryptose beef heart agar for 40 hours at 39°C and the mixtures were centrifuged after having been held at 5°C overnight The supernatant was discarded and the sediment was washed in saline centrifuged and resuspended in saline and merthiolate (1/7 500) to make up the volume of the original phenolized saline suspension, the bacterial content of which had been standardized

(*b*) *Alcohol and alum precipitated vaccine* Two volumes of 95% ethanol were added to the phenolized saline suspension as above and held overnight at 5°C 2 7 ml of a 10% sodium bicarbonate solution and 25 ml of a 4% potassium alum solution were added per 100 ml of volume and the mixtures were allowed to stand at 5°C for five hours The supernatant fluid was removed by centrifuging and the sediment resuspended in saline and held at 5°C for 40 hours Final treatment was the same as in case of the alcohol precipitated vaccine

Results in guinea pigs immunized with these vaccines and three weeks later either exposed from time to time during a month to the bites of infected

ıs or challenged by subcutaneous ınfection with a large dose of virulent gue bacilli were as follows

Vaccine	Flea infected guinea pigs tested	clinical plague	died	Subcutaneously infected guinea pigs tested	died
Alcohol precipitated	8	8	0	10	2
Alcohol alum precipitated (divided dose)				10	4
Alcohol alum precipitated (single dose)	5	4	0	10	3
Army	8	7	3	10	6
Typhoid	8	7	6	10	9
—	15 [a]	13 [a]	11 [a]	12 [a]	9 [a]

a Controls

Wayson and his collaborators expressed the opinion that alcohol and m precipitated vaccines were not superior to those obtained through ohol precipitation alone

Meyer [118] obtained the following results when challenging guinea pigs ich had been immunized with ordinary or precipitated vaccines by ious routes

gent used for killing organisms	Adjuvant	Number infected	Survival number	percentage
Phenol	None	29	5	17 2
	Alum	30	13	43 3
	Falba [a]	28	16	57 1
		30 [b]	0 [b]	0 [b]
Alcohol	None	30	7	23 3
	Alum	30	17	56 7
	Falba	30	24	80 0
		30 [b]	0 [b]	0 [b]
Formalin	None	40	10	25 0
	Alum	37	20	54 0
	Falba	37	33	89 7
		40 [b]	0 [b]	0 [b]

a " Falba is a mixture of oxycholesterin and lanolin
b Control animals

It will be noted that remarkably good results were obtained with malin killed and Falba precipitated vaccines and to a slightly lesser gree also with those killed by alcohol and precipitated with the aid of ba

(8) *Chemically prepared extracts of* P pestis As has been noted before ge 130) Kurauchi & Homma [101] were able to immunize guinea pigs, rats, d monkeys satisfactorily against plague infection with their specific uble substance ' particularly if this had been absorbed by metallic salts Meyer et al [1 1] recorded the results (given in table XII) which they tained with the various fractions of *P pestis* separated by them with aid of chemical procedures

TABLE XII RESULTS OF DOSING DIFFERENT ANIMALS WITH VARIOUS FRACTIONS OF PASTEURELLA PESTIS

Antigen		Guinea pigs		White rats		Cotton rats	Monkeys
		Dosage (mg)	Result [a]	Dosage (mg)	Result [a]	Result [a]	Result [a]
Fraction 1A		1 5	0 20	—	—	—	—
	alum	1 5	0 20	—	—	—	—
Fraction 1B		1	0 17	0 07	14 19	1 3	10 10
	alum	1 5	1 [illegible]0	0 07	16 18	6 10	—
Insoluble residue		[illegible]5	10 20	0 3	7 [illegible]0	0 10	10
	alum		16 20	0 35	19	—	—
Insoluble residue		1	13 20	—	—	—	—
	alum	1	19 [illegible]0	—		—	—
Formalin killed suspension		1	10	0 35	17 19	5 10	8 10
	alum	1	8 10	0 35	18 18	9 10	—

[a] The first figure in these columns indicates the number of survivors after challenge infection the second the number of animals used

It will be noted that the best results in guinea pigs were obtained with alum precipitated preparations of the water insoluble residue but that a formalin killed and alum precipitated vaccine also proved quite satisfactory In white rats on the other hand good success was obtained with fraction IB as well as with formalin killed suspensions of *P pestis* while the insoluble residue gave indifferent results

The vaccines obtained with the aid of bacteriophage will be dealt with later on

Live Avirulent Vaccines

Although some of the pioneers particularly Kolle & Otto [9] had shown that experimental animals including guinea pigs could be rendered immune to virulent plague infection through prophylactic administration of *P pestis* strains the virulence of which had been lost spontaneously or had been abolished by artificial means Strong [196] in Manila seems to have been the first modern worker who took practical advantage of live avirulent plague bacilli to immunize 900 human beings Since the disease was not present in Manila at the time the actual value of the method could not be assessed but it was significant that the serum of those inoculated was found to contain agglutinins and was also apt to protect experimental animals against challenge infection with *P pestis*

However, the history of inoculation with live avirulent plague vaccine may be said to have really begun in 1934 when *Girard & Robic*[67] and Otten[1 0] paved the way for the large scale use of this prophylactic in Madagascar and Java respectively The great success obtained in these two areas led to the introduction of this type of plague vaccination into other countries such as Argentina (Savino[161]) the Belgian Congo (Devignat[31]), Brazil (Goobar[67]), French West Africa (Rotman[15]), Tunisia (Magrou[110]), and the Union of South Africa (Grasset[69])

Girard & Robic s EV strain

The properties of the strain described by Girard & Robic,[63] and much used in Madagascar and elsewhere for human inoculation, are as follows

The EV strain had become rather avirulent while being subjected for five years to monthly agar subculture at 16° 20°C after that time proving innocuous to guinea pigs and rabbits when administered percutaneously, subcutaneously, conjunctivally, or by feeding Subcutaneous injection of guinea-pigs was apt to produce a local induration which disappeared in about a week without involving the regional lymph nodes (Girard & Robic[6]) Intraperitoneal injection of one third of a slant, or more, of this strain was apt to produce in guinea-pigs or rabbits a fatal peritonitis terminating in septicaemia, but the bacilli isolated from the blood were avirulent If lesser though still considerable, doses were given intraperitoneally and the animals were sacrificed from the 5th to the 15th day after injection nodules were found to be present in the spleen, and more rarely in the liver which did not contain the bacilli This process was accompanied by spleen hypertrophy which disappeared about the 20th day after injection

Girard & Robic laid great stress upon these reactions because they assumed that the antigenic potency of their strain was due to preservation of some of its virulence and toxicity In a later publication Girard & Radaody Ralarosy[61] also recorded that, in contrast to non immunogenic avirulent strains, the EV strain possessed invasive powers, subcutaneous administration of a dose of 1,000 million leading to the appearance of the bacilli after 40 hours in the blood, towards the 4th day in the spleen, and somewhat later in the liver The bacilli disappeared in the same order within two days after injection from the blood and within 11 and 13 days respectively, from the spleen and liver

At the same time however prophylactic administration of the EV strain protected guinea pigs against challenge with enormous doses of virulent plague bacilli by various routes including the bite of infected fleas and intratracheal infection

While *R rattus* behaved in much the same way as guinea pigs, mice and white rats being toxin sensitive, could not tolerate large doses of the EV strain They could, however, be satisfactorily immunized with smaller doses

Otten s avirulent strains

For his early work Otten[131,134] used the Tjiwidej strain named after the place where it had been isolated from a rat in 1929 This strain had lost its virulence spontaneously after having been kept in serum agar stab cultures at 5°C for four months

Subcutaneous administration of even 5 ml of a broth culture of the Tjiwidej strain or of suspensions prepared from whole agar slants proved harmless for guinea pigs and *R rattus diardii* At the same time the strain even in single doses proved highly immunogenic for these rodent species which Otten considered most susceptible to plague

While fresh suspensions of the Tjiwidej strain seemed to possess little toxicity if administered subcutaneously rats inoculated intraperitoneally were apt to succumb to toxaemia apparently because this mode of administration led to a rapid liberation of endotoxin For the same reason 35% of rats which had been injected subcutaneously with suspensions of the Tjiwidej strain heated to 60°C succumbed within 48 hours to toxaemia Similar results were obtained by Anchezar[4] when heating suspensions of the EV strain for half an hour at 58°C

The Tjiwidej like the EV strain produced a nodule at the site of subcutaneous injection (Otten[134] Savino & Anchezar[16]) Although according to the latter authors no macroscopically visible reaction was produced in the spleen Girard & Robic[63] could prove its presence through histological examination The Tjiwidej strain possessed invasive powers Otten[134] finding live bacilli present for periods of up to one week at the site of subcutaneous injection as well as in the organs particularly in the spleen

As has been mentioned before Otten[134] experimenting with a number of other plague strains was able to isolate avirulent variants through single colony picking He emphasized most appropriately that in this way alone it was possible to produce an irreversible loss of virulence

One of the above mentioned strains which had been originally isolated during the 1920 1 pneumonic epidemic at Harbin yielded an avirulent variant possessing a higher immunogenic power for guinea pigs than the Tjiwidej strain which in its turn protected rats particularly well Consequently mixtures of these two strains were prepared for human inoculation but as the Harbin strain was rather toxic it had to be used in a smaller dosage When inoculation work was resumed after the second World War it was decided at the Netherlands meeting on tropical hygiene, 1948 (Nederlandsche Vereeniging voor tropische Geneeskunde)[118] to dispense with this strain and use the Tjiwidej strain alone

Comparing the latter with their own strain Girard & Robic[63] thought the Tjiwidej strain to be more attenuated They agreed with Otten that it protected rats better than guinea pigs while the EV strain had reverse properties

Strains used in the Union of South Africa

As stated by Grasset,[69] the live plague vaccine at first used in the Union of South Africa was so prepared as to contain equal proportions of the EV and Tjiwidej strains with a total concentration of 1,000 million organisms per ml Later, however, in place of the Tjiwidej strain an old strain of Rowland s, labelled K/120, was utilized in conjunction with the EV strain The strain K/120 was highly immunogenic for *R natalensis* as well as for guinea pigs and rats, while the EV strain did not protect the latter or multimammate mice quite so well if virulent South African strains were used for challenge infection (Grasset [0])

Strains used in other countries

While the EV strain alone was used in Argentina, the Belgian Congo, Brazil, French West Africa, and Tunisia Korobkova[97] established that guinea pigs were immunized particularly well by a combination of this and her strain 46 S, obtained from a virulent plague growth through bacteriophage action A single injection of a mixture of both strains or an initial inoculation with 46 S followed one week later by EV inoculation protected guinea pigs against challenge infection eight months later Only 78% of the animals inoculated with EV vaccine alone survived however, if challenged after the same interval of time

Views on immunity mechanism

Strong[196] considered the process of immunization produced by the administration of live avirulent plague bacilli as a true vaccination, the organisms multiplying in the tissues and their successive generations stimulating the production of corresponding groups of antibodies (Otten[131])

As has been stated before, in the opinion of Girard & Robic[63] the EV strain was immunogenic because it had not altogether lost its virulence and toxicity, possessing in contrast to non immunogenic avirulent strains invasive powers In fact in a later communication Girard[53] maintained that the avirulent and vaccinating strains are strains whose virulence is only weakened (and not attenuated which would imply that they are definitely fixed in that state) [b]

According to Girard, virulence had to be defined in relation to the animals, the mode of inoculation, and the dosage used Thus 10,000 EV organisms while innocuous if introduced subcutaneously or intraperitoneally, killed a guinea pig when given intracerebrally Girard postulated that the immunity engendered by inoculation with live avirulent plague strains was of a cellular rather than a humoral category and maintained that this assumption was supported by the work of Pokrovskaya & Kaganova[118] who claimed to have enhanced the defence mechanism of the lung against

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[b] les souches dites « avirulentes » et vaccinantes ne sont que des souches de virulence *affaiblie* (et non atténuée ce qui signifierait qu elles sont définitivement fixées dans cet état)

plague infection through the administration of live avirulent vaccines by inhalation As stated by Girard the antigenic properties with which the immunizing power of the avirulent strains was associated could become degraded the factors known to bring about this process were frequent subcultivation at 37°C and temperature variations to which the strains were subjected during transport or storage Robic[153] had shown that the virulence of the strains could be restored to a certain degree in the laboratory but in the opinion of Girard this had never been observed under natural conditions

In connexion with this last mentioned claim attention should be drawn to Devignat s recent observation [1] of a slight and temporary increase in virulence of the EV strain he used for vaccine preparation He states that

(*a*) A guinea pig inoculated subcutaneously with 2 ml of a batch of EV vaccine died five days later with typical autopsy findings yielding cultures of the strain from its organs

(*b*) The vaccine batches available at the time produced more marked local and general reactions in man than previous lots As noted before (page 120) Devignat successfully used his method of bubbling air through broth cultures of the strain to suppress this slight increase in virulence

Otten[131] refuting the view that the immunizing action of live avirulent plague strains was associated with their invasive powers ascribed the superiority of live over killed vaccines to differences in the antigenic pro perties which while deteriorating during preparation of the latter vaccines remained intact in the former Jawetz & Meyer[82, 8] also maintained that the immunogenic activity of avirulent plague strains was a function of their antigenic make up and not of their invasive or pathogenic power

Finding a close correlation to exist between a high fraction IB content of avirulent plague strains and their power in small doses of protecting mice against challenge infection as well as of stimulating the formation of anti bodies in monkeys and man Meyer et al[121] concluded that the immunogenic activity of avirulent plague bacilli depended in part upon their antigenic make up At the same time however these workers sharing the views of Strong maintained that

> there is every reason to believe that the high degree of immunity is dependent upon persistence of the antigenic agent in susceptible cells of the body and not merely on the intactness of the antigenic components

Recent work by Walker et al[01] confirmed the view that avirulent plague strains may engender immunity in two different ways As sum marized by these observers

> Those strains which are effective in stimulating development of immunity in mice may be the ones that even though incapable of extensive multiplication are rich in soluble envelope antigen or may be the ones that although less productive of envelope antigen have the capacity to multiply and to attain numbers adequate to provide neces sary quantities of envelope antigen

Preservation of antigenic potency

To preserve the antigenic potency of avirulent plague strains destined for vaccine manufacture Girard [59] stressed the necessity of keeping them constantly at a temperature of 2°-4°C and *of subcultivating them not* more frequently than once a year He added that it had been possible in this way to keep the EV strain intact for 14 years

Storage

As maintained by Otten [133] and Girard [59] live avirulent plague vaccines cannot be stored for prolonged periods but must be used within a maximum of one month

Otten [134] noted in this connexion that, if kept at 5°C, his vaccine remained potent for several weeks Even at room temperature (23°C) the potency decreased only slightly within this time but the number of bacteria diminished and simultaneously the toxicity of the vaccine increased Tumansky [198] even maintained that live avirulent plague vaccines did not deteriorate for 16 months if kept at −13°C

Grasset, [0] while in agreement with these views stated that lyophilization of live avirulent plague vaccines considerably increased their keeping properties Experimenting with multimammate mice, he found that lyophilized samples, even if kept in the refrigerator for two years, lost little of their potency Cultures from these samples showed some delay in growth, but subcultures developed well

Experiences recorded by Girard [53, 59] in 1939 and again in 1948 regarding the lyophilization of live avirulent vaccines were unsatisfactory He found that lysis of the avirulent plague bacilli in normal saline occurred very slowly so that even after two years viable organisms could be found in suspensions with an original bacterial content of 1 000 million per ml Nevertheless, Girard considered it essential to use the EV vaccine rapidly so as to administer a maximum of viable avirulent bacilli However, this situation has now become altered in so far as to judge from a report published in 1952 [5] satisfactory laboratory experiences have been made with further samples of lyophilized EV vaccine prepared by Girard

Comparative Potency of Killed and Live Avirulent Vaccines

The laboratory evidence available in regard to the comparative value of killed and live avirulent vaccines, with which alone we are concerned at the present juncture has been the subject of much and sometimes even acrimonious, debate

To compare the results obtained by numerous investigators with either killed or live vaccines would be most difficult not only because they worked with differently susceptible species or races of animals and challenged

them with differently sized infective doses but mainly because they used methods of different accuracy to standardize the dose.

It would be expected that the results of comparative tests made by the same worker at the same time would be of great value for assessing the merits of killed and live vaccines respectively. Unfortunately, however, the results obtained by such comparative tests are quite often not really comparable: the workers interested in killed vaccines matched these against live avirulent preparations the potency of which was not as high as that of the live vaccines actually used in areas like Java and Madagascar, while the advocates of live vaccination tested their products against killed ones less carefully manufactured and standardized than the vaccines now issued by, for instance, the Haffkine Institute.

For these reasons it seems advisable, instead of comparing the experiments made by individual workers, to weigh on the one hand the total evidence available in regard to the killed vaccines and on the other that brought forward in favour of the live avirulent vaccines. If this is done, no doubt can exist in an impartial mind that, if carefully prepared and fully potent products are used in proper dosages and adequate challenge tests are made, equally good laboratory results can be obtained with both categories of plague vaccines. It follows that the question as to which of them should be used for man cannot be decided through experiments with laboratory animals but must be settled according to observations made in the prophylaxis of human plague—a problem which will receive full attention in a later chapter.

Onset and Duration of Active Immunity to Plague

Rowland [159] established that immunity produced in white rats by plague nucleoprotein evolved rapidly, being distinctly evident after 24 hours and reaching its maximum on the third day. Stevenson [191] (see also Stevenson & Kapadia [19]), inoculating a large number of Madras rats with Haffkine's vaccine, found that immunity began to develop within a few hours and rose till the second or third day. On the other hand it is interesting to note that according to the German Plague Commission [11] immunity in monkeys appeared much later, being slight on the fifth and reaching its maximum on the seventh day. As pointed out by Petrie [179] these results were consistent with the observations of Brooks on the development of opsonins: in the white rat the maximum response occurred on the first or second day and in man on the fifth or sixth day.

In the case of the live avirulent plague vaccines, immunity became apparent, according to Girard & Robic [64], five to ten days after inoculation. Otten [121] however states that it appeared after five to seven days and became maximal after two to three weeks.

The statements made by a few observers that, in experimental animals as well as in man, plague inoculation was followed by a "negative phase during which the inoculated individuals were particularly susceptible to the infection, will be dealt with in connexion with the problems of plague prophylaxis As will be shown, fears of this kind are unwarranted

Rowland[158] found, in the course of the above mentioned experiments, that the immunity conferred by his nucleoprotein was unimpaired at the end of three months and still appreciable at the end of five months Dieu donne & Otto,[31] dealing with this problem in a general manner, stated that no exact information existed as to the duration of the immunity produced in animals by inoculation with killed cultures, but took it to last for months, thus, according to Kolle rats protected by a single injection still showed after five months a marked immunity, even when infected intraperitoneally

These observations have been corroborated by experiments made with live avirulent plague vaccines Otten[131] stated that the immunity produced in guinea pigs by administration of the Tjiwidej strain was still fairly satisfactory after six months but had markedly decreased after the ninth month The EV vaccine was found to produce in these animals an immunity lasting for about a year or even longer (Girard[59])

PASSIVE IMMUNITY

Even since Yersin, Calmette & Borrel[05] had demonstrated in 1895 that the serum of rabbits treated with killed *P pestis* cultures was apt to prevent or even cure infection in normal animals numerous workers have endeavoured to produce immune sera suitable for the treatment and prophylaxis of human plague The results of these labours will be appre ciated in a later chapter devoted to the clinical aspects of the disease (see page 409) It is appropriate here however, to describe the methods of pro ducing the sera and of assessing their value, and to define their properties

Production of Immune Sera

Animals used

While horses were the usual, they were by no means the only animals chosen for the production of plague immune sera, several workers preferring other smaller or larger domestic animals such as goats sheep, calves mules bullocks and buffaloes

Attention was also paid to the possibility of using the rabbit which as noted above was the animal chosen by Yersin and his co workers for their original experiments and which was continually used to produce immune sera for laboratory needs Naidu et al[126, 127] though obtaining excellent experimental results with rabbit sera considered the yield from this animal

insufficient for actual serum production However the use of rabbits for this purpose was again recommended by Korobkova [96] and by Jawetz & Meyer [81] In view of the fully satisfactory experimental results of the latter a concentrated rabbit immune serum for the treatment of human plague was actually produced in the USA (Meyer [117, 119])

Although the rabbit in contrast to the horse has the advantage experimentally of being susceptible to infection with *P pestis* in view of the great general suitability of the latter animal for serum manufacture it is not surprising to find that most workers continue to use it for the large scale production of plague immune sera Particularly noteworthy in this connexion is the new horse produced plague serum of the Haffkine Institute the efficacy of which is shown by the following comparative results obtained by Sokhey [177]

Type of	[illegible] ml
New Haffkine	0 05
Buffalo (Naidu et al)	0 30
Pasteur Institute	0 50
Lister Institute	0 50
Commercial product	No protection with 0 5

Antigens used

A whole series of antigens or combinations of different antigens has been used by the various workers bent on the production of potent plague immune sera

Generally speaking the pioneers in this field used either killed plague cultures alone or first these and then live virulent bacilli some however completed the process of immunization by administering also toxic filtrates of plague *cultures or similar products to their animals Some workers* relied on the latter alone for instance nucleoproteins were used by Lustig and others broth filtrates by Markl and Dean (Pollitzer [14])

Recently live avirulent plague bacilli were used in place of the above mentioned antigens (Girard, [50, 51] Robic [15] Pirie & Grasset [14, 113] Schutze [166] Savino & Anchezar [16] Jawetz & Meyer [81] Korobkova [96])

Using a combination of the above mentioned antigens Sokhey [177] prepared the new Haffkine serum by immunizing horses first with living avirulent plague cultures then with live virulent growths and finally with filtrates of broth cultures which had been incubated for three weeks at 27°C

The following special methods of immunization also deserve mention

(*a*) Joukov Verejnikov et al [89] reported good results in guinea pigs with sera obtained by immunization of horses with lysates of envelope cultures of *P pestis* or with isolated envelope substances

(*b*) Girard & Sandor [65] found that the serum of horses which had been hyperimmunized with plague anatoxin (toxoid) gave experimental

results identical with those obtained using sera which had been prepared with the aid of live avirulent but toxic plague bacilli

(c) Meyer [117] obtained potent antisera by the immunization of rabbits and monkeys with the crystalline fraction IB plague antigen

Concentration of sera

As reported by Russell [159] fractionation of the sera prepared by Naidu and his colleagues with different concentrations of sodium sulfate had shown that the protective power was vested in the globulin fractions and not in the albumin fraction While it would seem that no large scale practical advantage was taken of these findings, actual use has been made of concentrated plague immune sera introduced by Pirie & Grasset [131, 14] Their original procedure consisted of two fractional precipitations by means of sodium sulfate and isolation of the pseudoglobulin fraction which in their opinion contained the majority of the plague antibodies However, they afterwards used a one process method to obtain the euglobulin pseudoglobulin constituents which gave more satisfactory results

Meyer,[117, 118] experimenting with rabbit immune sera found that the antibodies were mainly contained in the gamma globulins which were consequently isolated for the preparation of concentrated serum

Methods for Assessing the Potency of Immune Sera

Although as summarized by Jawetz & Meyer,[84] tests for measuring the potency of plague immune sera had been proposed by numerous investigators, most of the earlier methods were unsatisfactory because at best they permitted merely a rough evaluation of the results

An accurate and fully reproducible biological test introduced by Sokhey [177, 181] was based upon the use of the highly and uniformly susceptible Haffkine Institute inbred white mouse and of the standard infective dose as used also for the evaluation of plague vaccines The principle of the test was to determine the minimum of a given serum which would protect half the number of mice used against the standard infective dose given simultaneously Details of the test were as follows (Sokhey [181]) for any given serum five graduated doses were decided upon after a preliminary test, so that the 50% end point protective dose fell about the middle of the selected series For each dose a batch of five mice was used Both the serum and the standard test infective dose were given at the same time subcutaneously, but in different parts of the abdominal wall

Any mouse dying during an observation period of 30 days was examined for evidence of plague infection by smear and cultural examination At the end of the observation period all survivors were killed and likewise

examined The same held good of the ten controls used for each test which, given the infective dose only were usually dead by the ninth day

To avoid inaccurate results each determination was made twice From the results of these two determinations the 50% end point was calculated according to the method of Reed & Muench or another suitable method A potent antiplague serum was required to have a minimum mouse protective dose of not more than 0 05 ml

The principle of a curative test introduced by Sokhey at the same time was to determine the minimum quantity of a given serum which would save a plague infected mouse when serum administration was begun after the development of bacteriemia (which with the standard infective dose used became apparent within 72 hours) Essential features of the test were

For any given serum five graded doses were decided upon so that the 50% end point curative dose fell about the middle of the selected series For each dose a batch of five or six mice was used The animals were first given the standard test infective dose and 72 hours afterwards the serum in four equal portions the first being administered intravenously and the remaining three subcutaneously at 24 hour intervals This determination was done twice to avoid inaccurate results and the mice were observed as described above On the results of the two determinations the 50% end point was calculated A suitable plague serum was required to possess a minimum mouse curative dose of 0 4 ml or less

Following up earlier work by MacConkey [109] and others, Jawetz & Meyer [81] proposed a mouse protective test based upon the use of a standard serum They defined as the provisional standard unit the amount of serum which would protect 50% of mice from death when 1 000 average lethal doses (a l d)—about 2 000 5 000 plague bacilli—were injected intra abdominally 60 minutes after administration of 0 5 ml of serum dilutions by the same route The potency determination of the unknown plague immune sera was carried out similarly to the assay of antipneumococcal and antipertussis sera

It should be noted that Meyer & Foster [1 0] utilized a similar mouse protection test for the measurement of protective serum antibodies in human volunteers inoculated with plague prophylactics

Together with their mouse protective test Jawetz & Meyer [81] recommended a technique for a toxin antitoxin neutralization test using for this purpose the toxin prepared according to the method described earlier (see page 122) For performance of the test saline dilutions of the sera in quantities of 0 5 ml were injected intra abdominally into white mice followed 30 minutes later by 0 3 ml of the toxic filtrates diluted in saline solutions For purposes of comparison an arbitrary scale was chosen indicating the relative capacity of a given serum to protect mice against a standard dilution of the test batch of toxin It was found most advantageous to test three twofold dilutions (1 4 1 8 and 1 16) of serum (ten

mice each) against a filtrate dilution of 1 5 (approximately 10 a l d) The mice were observed for 48 hours and the deaths recorded The results were expressed as the number of mice surviving over the total number of mice used per serum

Properties of Immune Sera

Schutze,[164] Bhatnagar[12] and Gheltenkoff[46] concluded from their serological studies that plague immune sera contained two kinds of anti bodies corresponding to the ' envelope ' and somatic antigens of *P pestis*

In order to study the antibody content of plague sera by immuno chemical procedures, Girard & Sandor[6] used a method of serum frac tionation devised by Sandor, with the aid of which it was possible to distin guish between the bacterial antibodies contained mainly in the euglobulins and the antitoxins present in the pseudoglobulins

Investigating two sera, prepared with toxic live avirulent plague bacilli and with plague toxoid respectively, Girard & Sandor were able to separate three fractions, namely (*a*) euglobulin I which showed no antitoxic activity, but possessed antibacterial, agglutinating and protecting properties (*b*) euglobulin II a comparatively inert lipo protein fraction and (*c*) pseudoglobulins representing the antitoxin and endowed with but feeble precipitating, agglutinating and protective properties

Studying this matter further, Sandor et al [160] found that the sera obtained from horses, which had been immunized intravenously with either live plague bacilli or toxoid contained large amounts of euglobulin I However in sera produced through subcutaneous administration of plague toxoid, this euglobulin was largely replaced by another fraction, called euglobulin IIA by the authors In contrast to the above mentioned sera those produced by the subcutaneous route possessed no flocculating or agglutinating properties While the protective action exerted by all the sera in mice experiments appeared to be vested in the pseudoglobulins as well as in the euglobulins euglobulin I if present in considerable quantities, was definitely more active than the pseudoglobulins

The classification of plague immune sera has been the subject of much debate As was mentioned on page 135 the serum of plague immune animals is unable to destroy *P pestis* in vitro or in vivo in the absence of phago cytic cells It must likewise be noted that plague immune sera even if obtained by administration of plague toxins (broth filtrates or the like) or by additional immunization with such toxins possess only moderate antitoxic value Petrie[138] maintained in this connexion that, for no obvious reason, plague antitoxin in high concentration is not easily produced in horses Gheltenkoff,[45] however, believed that this was true of some of the horses only, while others produced sera of a satisfactory antitoxic titre A careful selection of suitable animals was therefore essential

Be this as it might, it is generally agreed that plague immune serum fits neither into the class of bactericidal nor that of antitoxic sera, belongs like the anthrax and rinderpest sera to the group of "anti-infectious" sera. Meyer,[11] while stating that it is not exactly known to what the anti-infectious properties of plague immune serum are due, drew attention to the hypothesis of Petrie and of Jawetz & Meyer that it probably produces significant opsonization.

SERODIAGNOSTIC METHODS

Agglutination

Preparation of agglutinating sera

According to the usual procedure for the preparation of agglutinating sera, most plague workers used rabbits for this purpose and as a rule immunized them by the intravenous route.

Of the different antigens recommended by the various workers in this field, the following deserve mention.

To obtain an agglutinating serum Batchelder[13] used a normal saline solution containing 0.25% formalin to wash off the growth of plague bacilli on hormone agar containing 0.075% of 10% sodium sulfite. The resulting suspension, which became sterile when kept for seven to eight hours at room temperature, was well tolerated by rabbits even if large doses were given intravenously and produced, when administered twice, a serum with a titre ranging from 1:1280 to 1:2560.

To produce sera against virulent *P. pestis* and also against pseudotuberculosis bacilli for his serological studies, Bhatnagar[13] resorted to the use of silver nitrate as employed by Rainsford for the manufacture of TAB vaccine.

As noted already, Baker et al. obtained immune sera with good agglutinating properties when administering their fraction IA and IB antigens to rabbits. The use of a serum produced with the aid of the water-extractable protein of *P. pestis* was also recommended by Seal,[164] who stated that "it may be possible to prepare this antiserum directly with the water-extractable protein solution of the virulent plague strain freed from bacterial debris by filtration, i.e., without preparing it at one-third saturation with sodium sulphate".

When preparing agglutinating sera with live plague bacilli most workers preferred strains which had lost their virulence spontaneously or which had been rendered avirulent artificially. It would seem, however, that Wats et al.[80] used virulent strains to complete the immunization of rabbits which had been previously inoculated subcutaneously and then intravenously with graduated doses of plague cultures heat-killed by exposure to 55°C for 30 minutes. Greval & Dalal[73] reported satisfactory results

when making agglutination tests with the therapeutic sera produced in the Haffkine Institute with virulent plague cultures

Preparation of suspensions

The main difficulty in carrying out agglutination tests with plague bacilli is to obtain suitably uniform suspensions of the organisms Though numerous procedures have been devised to overcome this impasse (see summary by Pollitzer,[147] and also Ciantini [6]) Wats et al [170] again studying this problem found none of them fully satisfactory and therefore recommended the following method

Roux bottles, containing meat digest agar (pH 6 8) were sown with thick suspension of plague bacilli and incubated for four days at 27°C or 37°C according to the nature of the agglutinable antigen wanted The growth was then washed off with 20–30 ml of distilled water containing 1% carbolic acid The washings were poured into a sterile test tube containing glass beads and kept at room temperature (27°–29°C) for six hours with an initial shaking by hand for five minutes and an occasional shaking during the first two hours The supernatant homogeneous layer was then pipetted off and left in the refrigerator overnight, so as to allow coarse particles to settle down The stable portion obtained from the suspension was centrifuged and after the deposit had been washed in saline twice, it was re suspended in normal saline containing 0 25% carbolic acid 0 05% formalin was added if the suspension was produced in bulk for stock purposes

It would seem that the simpler procedures recommended by Bhatnagar [13] and Seal [167] are not as reliable as the method described above

As stated previously (see page 88) Devignat [30] had found that bubbling of air through broth cultures produced a rapid and homogeneous growth of *P pestis* Recently studying the problem of agglutination [3] he used suspensions obtained with the aid of this method, stabilizing them by adding one drop of formalin per 20 ml of the growths

Rapid agglutination tests

In addition to tube tests carried out in the classical manner several workers recommended slide agglutination tests for the rapid diagnosis of human plague Thus Panja & Gupta [135, 136] used the sera of patients in dilutions of 1 3 or 1 4 A mixture of plague bacilli obtained from a few different agar or blood agar cultures incubated for one to two days at 37°C was distributed into drops of the serum dilutions and the results were read after one minute Positive results were obtained in 15 out of 17 cases with a positive blood culture usually on the seventh day after onset of the disease sometimes earlier The two negative cases had been tested on the third and fourth day respectively In 11 bacteriologically negative cases agglutination proved positive within 4 to 14 days after onset 75 normal sera yielded negative results

It should also be noted that recently Tumansky[199] recommended Noble s rapid method of tube agglutination (see Pollitzer[117]) for the identification of plague cultures and for testing the sera of plague suspect rodents with the aid of known strains of *P pestis*

A method recommended by Menezes[116] for the diagnosis of rat plague likewise deserves mention 250 mg of the spleen or liver of the suspect rats were suspended in 20 ml of normal saline and mixed with equal amounts of 1 10 1 20 and 1 40 dilutions of immune sera raised against 37°C growths of *P pestis* The tubes were then incubated in a water bath at 40°C and readings were taken after two to three hours A positive reaction was manifested by a heavy deposit consisting of agglutinated plague bacilli

Menezes admitted that the test was negative when plague bacilli were scanty in the organs of the rats but stressed on the other hand that plague livers or spleens which had become putrid or had been kept for a month or even longer in a desiccated state or in glycerol still gave a positive result

Types of agglutination

While the existence of serologically different races of *P pestis* has been unanimously denied attention has been drawn to the occurrence of two distinct types of agglutination Wats et al [0] maintained that these differences depended on the agglutinable antigen and not on the type of antisera employed Bhatnagar[13] on the other hand ascribed them to the presence of two antibodies in plague immune sera

Carrying out agglutination tests with plague bacilli cultivated at room temperature (27°–29°C) and at 37°C respectively Wats and his co workers obtained different results as follows

Room temperature growths	*37°C growths*
Agglutination slow	Agglutination rapid
Flakes small and uniform	Flakes larger and of varying size
Sediment compact	Sediment voluminous
Sediment not easily dislodged small flakes seen in a clear fluid on shaking clumps take time to reform and settle	Sediment easily dislodged uniformly distributed on shaking clumps reform quickly (10 minutes) and settle in large masses

Wats and his colleagues made the following interesting findings when carrying out comparative absorption tests

(*a*) 37°C growths were capable of absorbing all agglutinins from the sera obtained by immunizing animals with either 37°C or room temperature growths

(*b*) Room temperature growths on the contrary were capable of removing all antibodies only from sera raised against them and not from sera prepared with the aid of 37°C growths

(*c*) 37°C growths when heated at 100°C for one hour reacted more or less like room temperature growths

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(*c*) 37°C growths when heated at 100°C for one hour reacted more or less like room temperature growths

Obviously, therefore, the 37°C growth of *P pestis* possessed an additional antigen which was destroyed by heat and was absent in cultures incubated at room temperature

Bhatnagar,[13] studying the agglutination of plague bacilli possessing an 'envelope' and of plague and pseudotuberculosis strains which were devoid of an "envelope", with the aid of seri raised against virulent avirulent immunogenic, and avirulent non immunogenic plague strains also noted the presence of two types of agglutination as follows

Envelope agglutination	*Somatic agglutination*
Forms slowly	Forms slowly
Settles slowly	Settles slowly
Supernatant remains clear	Supernatant remains clear
Sediment voluminous flakes large woolly in character and varying in size	Sediment scanty flakes small uniform and gritty in character
In lower dilutions sediment easily dislodged and producing a shimmer in the serum suspension mixture in higher dilutions definite woolly particles visible	Sediment easily dislodged and becoming similar to saline control on shaking in all the dilutions of serum

It will be noted that there was a great deal of resemblance between Bhatnagar s somatic agglutination and that of room temperature growths observed by Wats and co workers on the one hand and between the envelope agglutination and that of 37°C growths on the other

Specificity of agglutination tests

Summarizing the evidence available up to 1936, Pollitzer[14] reached the conclusion that agglutination tests with plague and pseudotuberculosis immune sera as practised up to then formed no exact means of differentiating between these two bacterial species He added however, that by a judicious application of the newer knowledge upon the antigenic structure of these two germs a satisfactory method might be evolved

Bhatnagar[13] reporting in 1940 on investigations of this problem stated that (*a*) pseudotuberculosis immune sera did not react *with plague* bacilli and (*b*) pure envelope sera obtained through absorption of plague immune sera with *P pseudotuberculosis* strains no longer agglutinated pseudotuberculosis bacilli However as will be gathered from table XIII which summarizes observations recently recorded by Seal[169] this worker was not in full agreement with Bhatnagar s first claim

To judge from these findings a serological distinction between *P pseudotuberculosis* and virulent or avirulent protective plague strains can be made with most of the above mentioned methods of examination (numbers 2 to 6) but only a pseudotuberculosis serum absorbed with *P pestis* is satisfactory for a distinction between pseudotuberculosis bacilli and avi

TABLE XIII SEROLOGICAL RELATIONSHIP BETWEEN P. PESTIS AND P. PSEUDOTUBERCULOSIS

Sera produced with	*P. pestis* Virulent and avirulent protective	*P. pestis* Avirulent non-protective	*P. pseudotuberculosis*
(1) Virulent *P. pestis*	+	+	+
(2) Virulent *P. pestis* and absorbed with *P. pseudotuberculosis*	+	0	0
(3) *P. pestis* boiled for ½ hour	0	+	+
(4) *P. pseudotuberculosis*	0		+
(5) Water extractable protein of *P. pestis*		0	0
(6) *P. pseudotuberculosis* and absorbed with *P. pestis* or *P. pestis* boiled	0	0	+

According to Seal

rulent non-protective plague strains as well. In actual practice one is not likely, however, to meet with the latter strains.

Vargues[400] recently recorded that the plague hyper-immune serum prepared in the Pasteur Institute at Paris had the property of agglutinating suspensions of *Salmonella*, *Brucella* and *Proteus* in the cold. However, the agglutination titre was not higher than 1/320, so that this phenomenon could be easily distinguished from the agglutination of *P. pestis* taking place at high titre at 37°C.

In the opinion of Vargues, the presence of euglobulin I in the immune serum was responsible for this unspecific agglutination in the cold. He stated in this connexion that while the euglobulin I is easily soluble in normal saline at ordinary temperatures, it precipitates after the solutions are cooled to + 4°C. The euglobulin is then apt to affix itself to inert corpuscles such as bacteria or horse erythrocytes and to agglomerate them.

Scope of agglutination tests

Advantage may be taken of agglutination to test unknown strains with a plague serum of established potency and to examine the sera of suspects with the aid of known cultures. As has been noted, Panja & Gupta[13, 136] obtained good results with rapid slide tests and the value of agglutination for the diagnosis or retrospective diagnosis of human plague has also been endorsed by other recent observers, particularly by Favarel[37, 38]. This worker came to the opinion that, except in the case of recently inoculated individuals, agglutination even at low titres was

significant Agglutinins appeared as a rule about the seventh day of illness Two persons who had been cured showed negative reactions after nine and ten months respectively

It is particularly noteworthy that, as recorded by Favarel and also by Huang et al [81] and Greval,[72] treatment with sulfonamides or streptomycin though apt to lead to negative bacteriological results did not adversely affect those of agglutination tests

Haemagglutination

Keogh et al [91 92] and some other workers recently recommended an indirect method of agglutination, based upon the observation that some micro organisms have a component which can be adsorbed to erythrocytes and that erythrocytes thus sensitized are agglutinated by immune sera specific to the micro organisms in question

The possibility of using this method of haemagglutination in the case of the plague bacillus has been studied by Amies [3 191] and by Chen [3]

Amies [3] thus described the technique he used for this test

Both rabbit and horse anti plague sera were employed and the red cells were obtained from human sheep and guinea pig blood The standard procedure was to use sheep cells The preliminary absorption [to remove anti species antibodies potentially detrimental to the erythrocytes from the sera] was carried out by adding one volume *of packed cells to 10 volumes of inactivated serum and centrifuging one hour later* A 0 1 per cent solution of the purified envelope antigen was added to another portion of the erythrocytes in the proportion of one volume of packed cells to 10 volumes of antigen After one hour s contact at room temperature the cells were collected by centrifugation and washed 3 times in physiological saline The agglutination test was then *performed by adding 0 1 ml of the packed sensitized cells to 1 0 ml of serum in a dilution* series from 1 in 10 to 1 in 10 000 or higher The tubes were held in a water bath at 37° for one hour and then placed overnight in the refrigerator the results being red on the following morning

In the opinion of Amies the haemagglutination test was unlikely to be of much value as a diagnostic procedure because of the slow development of immunity in plague but was possibly useful for confirming retrospectively a diagnosis made on clinical grounds only besides being of *unquestionable value for following the development of antibodies in animals* undergoing immunization

Chen [23] established the interesting fact that the antigen responsible for haemagglutination, though present in old broth cultures of *P pestis* as well as in extracts of killed and dried plague bacilli and in the *alcohol* precipitated envelope antigens, was absent in the protein fractions obtained with the aid of ammonium sulfate The evidence accumulated by Chen indicated that this antigen which was probably of a polysaccharide nature was of no importance as a protective agent in plague infection

Precipitation

While the opinions held by earlier workers (see Pollitzer[14] for summary) regarding the value of precipitin tests for the diagnosis and differential diagnosis of plague had been divergent *this method was recently recom*mended by Cambosu and by Larson et al (see chapter 5 p 243) for the rapid recognition of rat plague

The precipitation test with protein fractions of plague and pseudo tuberculosis bacilli devised by Seal[140] may be said to be of theoretical rather than of practical interest the more so because the reactions obtained with the aid of this method closely tallied with those produced by agglutina tion tests

Flocculation

Girard[54] established that especially in the case of patients with sup purating buboes of long standing good diagnostic results could be obtained by mixing five drops of the patient s serum with 1 ml of plague endotoxin (filtrates of broth cultures or preferably extracts obtained by repeated freezing and thawing) flocculation took place in positive cases Readings were taken after 30 minutes 3 hours and 24 hours

At the same time Girard was unable to confirm the claim of Gheltenkoff[46] that flocculation tests were useful for standardizing plague immune sera

Haemolysins

The evidence available on the reactions produced by *P pestis* in blood containing media is not only somewhat scanty but most contradictory

Korobkova[98] stated in this connexion that both plague and pseudo tuberculosis bacilli were endowed with haemolytic properties a clear zone appearing within 48 72 hours round colonies on agar plates which contained 5% of defibrinated rabbit guinea pig or horse blood The same phenomenon was observed on plates containing sheep blood but in contrast to the above mentioned media the sheep erythrocytes remained intact In liquid media containing 2% of blood haemolysis became com plete in five days

As noted by Korobkova filtrates of 12 day old plague cultures caused feeble lysis of sheep erythrocytes The haemolytic filtrates caused the death of white mice but not of rabbits The haemolysins present in plague cultures were not neutralized by plague immune or antihaemolytic sera and possessed no immunogenic properties

In contrast to the opinion of Bielonowski[15] Korobkova maintained that there was no significant relation between the haemolytic power and the virulence of plague strains She found however that smooth avirulent plague strains produced haemolysis more slowly than virulent growths

while rough avirulent strains reacted like *P pseudotuberculosis*, lysing rabbit, guinea pig, and horse erythrocytes even more rapidly than virulent plague strains

In marked variance with these statements Colichon [7] maintained that pseudotuberculosis strains as well as the pasteurellae sensu stricto failed to produce haemolysis on blood agar plates Although admixture of human blood was most suitable to show up this difference between *P pestis* and the above mentioned micro organisms Colichon stated that it was permissible to use instead horse rabbit guinea pig or grey rat blood

Wagle & Habbu (unpublished observations) cultivating five laboratory strains of *P pestis* and two pseudotuberculosis strains at 28°C and 37°C respectively on blood agar slopes prepared with human guinea pig horse and rabbit blood, were unable to confirm the findings of Colichon, haemolysis being absent in the case of both micro organisms Repeated passage of the plague strains through susceptible experimental animals (*Gunomys kok*) did not alter their behaviour on the blood agar slopes

The recommendation made by the WHO Expert Committee on Plague at its second session (1952) that this matter should be further studied deserves all the more attention because, as maintained by a few observers such as Stephan [193] and Seal [168] *P pestis* is capable of producing haemo digestion on blood agar plates

Complement Fixation

Summarizing the literature available up to 1936 Pollitzer [147] stat that the use of complement fixation tests for the purposes of plagu laboratory work had been recommended by several investigators

Thus Damperoff [9] tested plague immune sera with the aid of this method using either bacillary suspensions or extracts as antigens He found fairly constant and reliable results but could not establish a correlation between the complement fixing titre and the curative value of the sera in question

Moses [1 5] found in 25 out of 38 plague patients complement fixing antibodies and twice demonstrated plague antigens, in most of these instances the blood had been taken on the fifth day of illness In the experience of Shchastny [1 4] however, antibodies were present not earlier than in the second week

The method of complement fixation for the diagnosis of human plague was again recommended by Joltrain [86] and Simard [1 6] The former reported positive results in clinically uncertain cases in some of which agglutination tests had been negative No false positives were seen Dickie [33] confirmed the usefulness of the method but thought it of greater positive than negative value

Gryscz & Wagon (quoted by Dieudonné & Otto[31]) testing old plague infected tissues stated that complement was specifically deviated even when there was advanced putrefaction However, Piras[139] could not confirm this claim for he found the reaction to be positive up to the sixth day only and therefore considered it inferior to animal experiments

The usefulness of complement fixation tests for the diagnosis of human plague was again noted by Joltrain[87] in 1936—particularly in the case of convalescents or of patients with pestis minor

Mitin[1 3] trying out 40 different antigens found it most suitable to suspend plague bacilli grown on agar in distilled water and to shake the suspension for two to four days in the dark After centrifugation the supernatant fluid was pipetted off and phenol was added so as to obtain a concentration of 0 5%

Using his 40 antigens to make complement fixation tests with the sera of immunized and non immunized animals as well as with those of inoculated and non inoculated human beings Mitin obtained satisfactory results He reached the conclusion that the method could not be used to diagnose sisel plague because the sera of these rodents had anticomplementary properties However Kuznetsova & Dobrokhtova[10] claimed afterwards that the reaction was useful to assess the incidence of plague among the sisels

A new procedure for complement fixation tests with plague fraction I as standard antigen and specific high titre plague immune serum preferably such produced with fraction I as standard antigen was described by Chen et al [4] who gave the following examples for the usefulness of this method

(*a*) Determination of fraction I plague antigen in the bacilli or in tissue extracts of animals succumbed to experimental plague

(*b*) Detection of antibodies to fraction I in the sera of human convalescents and immunized animals

Chen and his co workers maintained that besides being of value for the retrospective diagnosis of human plague their method of complement fixation was apt to be of importance for the field diagnosis of wild rodent plague specially when the isolation of *P pestis* or the interpretation of the pathological lesions at autopsy is rendered impossible by contamination or decomposition

The question as to whether complement fixation tests are useful for differentiation between plague and pseudotuberculosis bacilli has been answered in different ways by different observers To judge from the investigations of Zlatogorov & Mogilevskaya is well as from those of Boquet & Dujardin Beaumetz (quoted by Pollitzer[147]) complement fixation tests were of value in distinguishing between plague and pseudo tuberculosis strains it should also be noted that Damperoff[9] obtained

negative results when making such tests with pseudotuberculosis bacilli and plague sera However, Shchastny [1 4] Mitin [123] and recently Haas [7] found that pseudotuberculosis bacilli merely gave less marked reactions than *P pestis* when tested with plague sera, and the extensive studies of Greval & Dalal [73] led to the same result

As far as the diagnosis of human infections is concerned, it is of no vital importance that complement-fixation tests are unreliable, or at least not fully reliable for differentiating between plague and pseudotuberculosis because cases of the latter disease in man are rare, invariably solitary, and usually show peculiar clinical features distinct from those of plague

BACTERIOPHAGE INVESTIGATIONS

As summarized by Harvey [78] in a comprehensive survey of the bacteriophage problem with particular reference to plague and cholera and confirmed by further investigations in the case of plague phages have been isolated from rather varied sources such as rat faeces and the stools of convalescents, rat lymph nodes and serum buboes or blood from plague patients or convalescents sewage and canal water It is of special interest that positive findings were not invariably restricted to localities where the infection was present

Thus Girard [48 49] detected an active lytic principle to *P pestis* in rats from a former plague focus in Antananarivo Madagascar, and postulated that a causal connexion might exist between the presence of phages in the rodents and the disappearance of the infection

Lepine & Bilfinger [105] examining rats at Athens at a time when an enzootic seemed to be absent found the serum of two out of 217 animals to be lytic for *P pestis*

Flu & Flu [4] (see also Flu [49]) repeatedly isolated from sewage and canal water at Leyden, Netherlands phages which were active against plague and pseudotuberculosis bacilli as well as against *Eschericia coli* and shigellae Flu & Flu assumed that the phage strains had originated from rat intestines where they had been adapted to *E coli*

Although as in the case of other infectious diseases far reaching claims have been made regarding the role played by bacteriophages in the development of a natural immunity against plague, it seems altogether unlikely that the influence they might exert in this direction is of general importance Discussing this problem Harvey [78] stressed that Avari in India had been unable to find phages active against *P pestis* in either Bombay or Madras rats

The question of to what extent the action exerted by bacteriophages on plague growths is specific in nature and how far, consequently observations made with the aid of these lytic agents are of differential diagnostic importance has been the subject of much discussion

FIG 17 P PESTIS COLONIES ON AGAR PARTIALLY LYSED BY PHAGE



The early workers though sometimes finding that the action of their phages was restricted to those plague strains to which they were adapted admitted the existence of polyvalent strains possessing lytic powers for all plague growths However they were unanimously of the opinion that the action of plague bacteriophages was specific as far as other bacterial species in particular *P pseudotuberculosis* were concerned Advier [1] established in this connexion that an agar surface treated with a plague phage ceased to be suitable for the growth of *P pestis* but continued to be favourable for the development of other micro organisms and recommended this method for the purposes of differential diagnosis

Bezsonova et al [12] working with two bacteriophages of wild rodent origin found them to lyse all 214 plague strains tested but to exert no action on 21 pseudotuberculosis strains They considered therefore the use of suitable plague phages as a subsidiary means for the differentiation of the two organisms

Girard [56] noted that plague phages were able to lyse pseudo tuberculosis strains to the same titre as pseudotuberculosis phages and later [57] also confirmed the observation of Flu & Flu that both these phages were active against dysentery bacilli while vice versa certain shigella phages lysed plague and pseudotuberculosis bacilli Nevertheless he maintained the differential diagnostic value of bacteriophage examinations made with

the aid of broth media pointing out that the different appearances of growth shown by plague and dysentery bacilli in the control tubes were so striking as to preclude diagnostic errors

In a paper published in 1947, Lazarus & Gunnison[103] reached the conclusion that bacteriophage tests did not constitute a means of clearly distinguishing between plague and pseudotuberculosis bacilli Further studies by Gunnison et al [74] showed, however, that it was possible to make such a distinction *with the aid of bacteriophage tests carried out at 20°C* The technique used by Gunnison and his co workers was as follows

Advier s phage strain was adapted on the one hand to an avirulent plague strain (P phage) and on the other to a pseudotuberculosis strain (PTB phage) The plague strains to be tested were first grown for 18 24 hours at a temperature of 18°–20°C in broth Dry agar plates were then implanted with the growths each of which was spread over a circular area, 2 cm in diameter, so that one dish could accommodate ten different growths After these had become dry, they were touched with a suspension of one of the two phages The plates were then incubated in an inverted position for 48 hours at 20°C and 37°C respectively Before carrying out the tests proper each phage was titrated against the culture to which it had become adapted by testing serial tenfold dilutions of the phage in question in the manner described above The plates used for the titration of P phage were held at room temperature those of PTB phage were incubated at 37°C The highest dilutions giving confluent lysis were recorded and for the final tests ten times this amount, the critical test dilution was used

The results obtained with 45 pseudotuberculosis strains and 52 plague strains (35 of which were virulent) were as follows

Type of phage	*Results*
PTB phage	Of no diagnostic value lysing most plague cultures at 20°C or 37°C and not lysing most of the pseudo tuberculosis cultures at 20° C
P phage at 37°C	Lysed many pseudotuberculosis strains and failed to lyse a few plague strains
P phage at 20 C	*Showed a specific action for plague strains*

Attempts to cure plague infected experimental animals with specific phages gave negative or even most disappointing results as a rule the treated animals often succumbing more quickly to plague than the controls As convincingly shown by Pons [118] in the case of guinea pigs this phenomenon was due to the liberation of plague endotoxin by the bacteriophage which thus became a pathogenic instead of a therapeutic factor

Joukov-Verejnikov & Favarissova [90] administering bacteriophage simultaneously with plague infection to guinea pigs found that the animals so

treated died at the same time as the controls The two workers maintained that the bacteriophage and the plague bacilli could co exist in the animal body without influencing each other and noting that in vitro addition of normal serum to a mixture of these two agents prevented lysis expressed the opinion that the absence of phage action in vivo might have been due to phenomena of a colloidal nature

Though occasional successes have been recorded the results obtained when treating plague patients with specific phages were as a rule as dis appointing as those in experimental animals Moreover this mode of plague treatment has become altogether obsolete in view of the availability of new therapeutic substances the action of which is by far more constant as well as most markedly more effective

Experimenting with different animals (grey and white mice white rats and guinea pigs) Advier[1] found that injection of a mixture of plague bacilli and bacteriophage if tolerated by the animals produced a solid immunity against subsequent plague infection Compton[9] who had obtained identical results maintained that the immunity thus produced did not result directly from the action of the bacteriophages but was due to the liberation of immunogenic substances from the bacilli

To test this hypothesis Compton used phage preparations treated with formaldehyde The filtrate obtained by passing the lysates through L_5 Chamberland candles was incubated for five days to ensure sterility Then formalin was added in the proportion of 0 4% (0 16% formaldehyde) and the mixture was heated in a vaccine bath at 60°C for one hour It was then kept in the incubator for 18 days before use

Compton claimed that better protection in experimental animals resulted from subcutaneous administration of this specific phage lysed vaccine than from three inoculations with untreated bacteriophage

Flu who dealt in numerous publications with the same problem described in 1933 the preparation of lysate as follows[41]

> A suspension of a 24 hour old broth culture of virulent plague bacilli containing 5 000 million organisms per ml was mixed with 2 % active phage and incubated at 37 C for 36 hours The partially lysed suspension was filtered through cotton wool shaken up with an excess of chloroform and again left for 12 hours at 37 C The fluid was then carefully decanted from the chloroform carbolized to 0 5 % allowed to stand for five days at room temperature and then tested for sterility Before use the fluid which was not quite clear had to be shaken

Flu stated that even one injection with the vaccine lysate protected 66% of white rats against infection with 10 000 m l d of plague bacilli whereas three inoculations protected 91% of the animals He emphasized the necessity of using concentrated suspensions of virulent bacilli for pre paration of the lysates alleging that the failures reported by other investi gators were due to the fact that they had worked with products poor in dissolved bacterial substances though rich in phages Flu s lysate remained

potent when kept for four months at room temperature Lazarus & Gunnison [103] confirmed that phage lysates of *P pestis* were effective in protecting mice against plague infection, adding that the use of such preparations 'offers a number of interesting possibilities for human vaccination '

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Chapter 4

PATHOLOGY

BUBONIC PLAGUE IN EXPERIMENTAL ANIMALS

Guinea pigs

Methods of infection

The highly susceptible guinea pig can be easily infected with plague by different routes indeed as first shown by Albrecht & Ghon [1] mere rubbing in of infectious material into the shaven depilated or even quite intact skin is ordinarily sufficient to produce a fatal infection

A few workers such as Wayson & McMahon [117] preferred intracutaneous administration of plague material to the above described procedure of cutaneous infection

Subcutaneous infection of guinea pigs was effected not only in the usual manner with the aid of a syringe but also by introducing a needle dipped into the infectious material under the skin or by putting the material to be examined into a skin pouch (Hauttasche) (Maassen [68])

Two other procedures of practical importance are intraperitoneal and intra ocular infection the latter being performed either through instilla tion of the infective material into the conjunctival sac or according to Cornil et al [0 1] through subconjunctival injection

In place of these artificial procedures some workers used infected fleas to produce plague in their test animals

Choice of methods

A choice between the different methods enumerated above depends upon the nature of the experimental work to be performed It is obvious that only those among them which permit of the introduction of exactly measured doses with the aid of a syringe will be of real value for standard ization tests Subcutaneous infection is the procedure usually resorted to for work of this kind

FIG 18 PATHOGNOMIC SIGNS OF ACUTE PLAGUE IN A GUINEA PIG



Purulent reaction at the site of infection — a large groin bubo — subcutaneous congestion — congestion of the lungs — swelling and early fatty degeneration of the liver

FIG 19 PATHOGNOMIC SIGNS OF ACUTE PLAGUE IN A WHITE RAT



Left axillary bubo (<—) — subcutaneous congestion — pleural effusion of the lungs — enlargement and dome like appearance of the liver

While in animals belonging to this group the appearances at the site of inoculation did not differ from those described above the massive gelatinous periadenitis typical for type 1 was not invariably, though frequently present Spleen and liver were voluminous and friable and the former organ in particular was studded with whitish granules of pin head size which when examined histologically, proved to be foci of necrosis usually surrounded by a belt of reticular cells The lungs were congested as in type 1 but in some of the animals belonging to the second group disseminated foci of bronchopneumonia were present as well

Type 3 found in guinea pigs which having been partially immunized with vaccines or serum had survived for more prolonged periods

While voluminous infiltrations sometimes showing abscess formation were present at the site of infection no signs of a gelatinous periadenitis could be found in this type round the buboes The spleen and liver were much enlarged and showed the presence of large whitish nodes Similar nodes were also seen in the lungs In contrast to the other types particularly type 1 smears made from the organs of type 3 guinea pigs showed few or no plague bacilli

Devignat and his co workers considered the massive gelatinous periadenitis met with in experimentally plague infected guinea pigs as pathognomonic It has to be noted however that Haas[46] observed this sign in 2 out of 39 guinea pigs infected cutaneously or subcutaneously with a pseudotuberculosis strain isolated from a rat He laid stress on the constant presence of liver nodules in his animals claiming that nodules of an identical aspect were seldom encountered in plague infected guinea pigs

The observations made by Cornil et al [20, 21] on the morbid anatomy and histology of plague in guinea pigs and white rats which had been inoculated with varying dosages of the same *P pestis* strain are of great interest and value even though they are based on an examination of small series of these animals

Cornil and co workers distinguished between the following three types of plague in their experimental animals which had been inoculated subcutaneously intraperitoneally or subconjunctivally

Typ f inf t	*Exp e tal anim l*	*Pe od b t en i f t a d d th (days)*	*Lo l eacti a t b bo*	*Ch es in l t l org*
Hyperacute (forme sur aigue)	Guinea pigs	3		Congestion of spleen signs of parenchymatous degeneration—marked in liver and adrenals less prominent in kidneys and myocardium congestion of brain with small haemorrhages little sign of bacterial embolism
	Rats	2 2½	Slight changes	

FIG 19 PATHOGNOMIC SIGNS OF ACUTE PLAGUE IN A WHITE RAT



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Type 3 found in guinea pigs which having been partially immunized with vaccines or serum had survived for more prolonged periods

While voluminous infiltrations sometimes showing abscess formation were present at the site of infection no signs of a gelatinous periadenitis could be found in this type round the buboes The spleen and liver were much enlarged and showed the presence of large whitish nodes Similar nodes were also seen in the lungs In contrast to the other types particularly type 1 smears made from the organs of type 3 guinea pigs showed few or no plague bacilli

Devignat and his co workers considered the massive gelatinous periadenitis met with in experimentally plague infected guinea pigs as pathognomonic It has to be noted however that Haas [46] observed this sign in 2 out of 39 guinea pigs infected cutaneously or subcutaneously with a pseudotuberculosis strain isolated from a rat He laid stress on the constant presence of liver nodules in his animals claiming that nodules of an identical aspect were seldom encountered in plague infected guinea pigs

The observations made by Cornil et al [20, 21] on the morbid anatomy and histology of plague in guinea pigs and white rats which had been inoculated with varying dosages of the same *P pestis* strain are of great interest and value even though they are based on an examination of small series of these animals

Cornil and co workers distinguished between the following three types of plague in their experimental animals which had been inoculated subcutaneously intraperitoneally or subconjunctivally

Type of infection	*Experimental animal*	*Period between infection and death (days)*	*Local reaction and bubo*	*Changes in internal organs*
Hyperacute (forme sur aiguë)	Guinea pigs	3		Congestion of spleen signs of parenchymatous degeneration—marked in liver and adrenals less prominent in kidneys and myocardium congestion of brain with small haemorrhages little sign of bacterial embolism
	Rats	2 2½	Slight changes	

Type of infection	Experimental animals	Period between infection and death (days)	Local reaction and bubo	Changes in internal organs
Moderately acute (forme aigue moyenne)	Guinea pigs	6 7	Local necrosis and suppuration (suppuration necrotique)	In addition to the above described process of degeneration necrotic foci due to bacterial embolism were present not only in the spleen, but also in the liver lungs and adrenals i e in organs possessing in contrast to the kidneys myocardium and brain an ample supply of venous blood
	Rats	3½		
Subacute (forme aigue prolongee)	Guinea pigs	12	Bubo suppurative adenitis in subcutaneously infected animals	
	Rats	5		

Considering these findings, Cornil and his colleagues formed the opinion that plague in their animals evolved in three stages (*a*) a phase of local reaction quickly followed by (*b*) a stage of toxaemia (septico toxemie diffuse) which led to degenerative changes in the internal organs, and (*c*) a final stage characterized by septico pyaemic metastases produced by bacterial emboli in some of the internal organs, which was reached only in animals surviving for comparatively longer periods

A peculiar type of plague in guinea pigs experimentally infected with plague strains from the Andes region of Ecuador was described by Macchiavello & Uriguen [7*] the animals in question showing in addition to regional buboes, marked involvement of the pelvic aortic lymph nodes as well as conspicuous often necrotic, lung lesions Macchiavello & Uriguen ascribed the appearance of these features to peculiarities of the plague strains, which seemed to be endowed with marked invasive power but little toxicity They suggested that the strains might have been antigenically different from typical plague bacilli met with elsewhere

White Rats

Methods of infection

While some workers considered cutaneous inoculation of white rats a suitable procedure for plague laboratory work most observers reached the conclusion that this method did not give quite as constant results with these animals as with guinea pigs A much recommended procedure is, however, that of pricking the root of the tail of white rats with an injection needle which has been dipped into the material to be tested One advantage of this procedure is that it may be easily and safely carried out in the glass jars in which the rats are kept before and after infection the ordinary cover is temporarily replaced by a wooden one with a hole in the centre (Impfdeckel "), with the aid of a long forceps or clamp the tail alone is drawn out and the inoculation is made (Kister quoted by Dieudonne & Otto [2])

Kolle & Otto (quoted by Dieudonné & Otto [6]) found this method useful when dealing with contaminated materials and also claimed that with its aid negative results were obtained with certain rat pathogenic bacteria which proved fatal when injected subcutaneously

Intraperitoneal infection of white rats though as a rule producing no marked gross changes in the peritoneal cavity, is a useful method because unless an overdose of the infective material has led to rapid death from toxaemia numerous plague bacilli may usually be found in the scanty and serous peritoneal exudate as well as at other sites especially in the spleen

In the past great attention has been paid to feeding experiments with laboratory rats because it was believed that the free living rats usually contracted infection per os Several investigators showed that rats may become infected in the laboratory when fed with plague cultures or carcasses and may develop mesenterial buboes However these findings were in strict contrast to those made under natural conditions Moreover, as shown by Kolle & Otto (quoted by Dieudonne & Otto [6]) even in the laboratory, infection by feeding deserved but little attention on account of the somewhat inconstant results

Kolle & Otto expressed the same opinion in regard to conjunctival infection of white rats However this method was recommended by the German Plague Commission [32] even for little virulent and contaminated material

Autopsy findings

As confirmed by the above mentioned observations of Cornil et al [0] [1] the autopsy findings in experimentally plague infected white rats (see fig 19) on the whole rather resemble those met with in guinea pigs Eastwood & Griffith [47] considered general redness and engorgement throughout the ventral subcutaneous tissues as features constantly and conspicuously present in white rats which succumbed to subcutaneous plague infection

Susceptibility to pseudotuberculosis infection

A point of great importance is the reaction of white rats to infection with *P pseudotuberculosis* Since in the experience of most workers these animals proved resistant to such inoculations non pathogenicity for white rats was considered as one of the cardinal features distinguishing pseudo tuberculosis from plague bacilli

The recent observations of Haas [46] were on the whole in agreement with this contention Though his pseudotuberculosis strain had been isolated originally from a *Rattus norvegicus* it showed little pathogenicity for laboratory rats four out of five such animals inoculated with it remained healthy two of them had apparently become resistant to subsequent plague infection The fifth rat which had been injected sub

cutaneously, was killed two weeks after infection It showed a small ulcer at the site of inoculation and some nodules in the spleen Smears from this organ proved negative, but a guinea pig infected with material from it succumbed to pseudotuberculosis 27 days after subcutaneous inoculation

However, since a few observers found pseudotuberculosis strains definitely pathogenic for white rats (Bezsonova[11]), it would not be wise to classify otherwise dubious strains as plague solely because they are able to kill such animals Certainly, however, a suspect strain which is pathogenic for guinea pigs, yet spares white rats, is not one of plague

White Mice

While in the past it was generally maintained that white mice were rather resistant to experimental infection with *P pestis*, it has now been established that certain inbred races of this species are eminently suitable for plague laboratory work Sufficient evidence regarding this point has already been brought forward (see page 117)

White mice which have been inoculated with plague material show, as a rule no features pathognomonic for this infection at autopsy Spleen enlargement and other non specific signs of a generalized infection may however, be met with in such animals It is important to note that they are susceptible to pseudotuberculosis infection (Poppe[99])

Rabbits

Although, as recorded by Taylor,[113] certain races of rabbits may prove fully satisfactory, generally speaking these animals are not uniformly susceptible to plague not uncommonly showing a resistance to virulent infection As a rule, therefore, the use of rabbits for plague diagnostic work is not to be recommended the less so as they are fully susceptible to pseudotuberculosis infection As has been noted, however, rabbits are indispensable for serological work and are at the same time excellent for the production of plague immune sera

Free-living Rodents

As has been stated before, free living rats have been used on a large scale for plague laboratory work in the Haffkine Institute In south east Russia ample advantage was taken for the same purpose of local sisel species, and in Africa of multimammate mice These examples suffice to show that the use of such animals for the various needs of plague laboratory work is legitimate provided that the following precautions are taken

(1) If the rodents in question have been received from a region where plague is endemic, due care must be exerted to ascertain that they are

free from the infection It may become necessary to quarantine the animals for some time but this may rather bias their usefulness since some of the species concerned particularly commensal rats are apt to show a considerable mortality in captivity even when caught healthy They also tend to mutilate or even partly to devour the carcasses of their dead mates which may render postmortem examinations difficult

(2) At the same time it must be ascertained whether the free living rodents to be used for experimental purposes are fully susceptible to plague It has been noted in this connexion that the Haffkine Institute had to send for its rats across India to Madras a plague free city with a rat population not resistant to the infection

(3) It is imperative to free the animals as soon as they are received from fleas and other external parasites The method of dipping the rodents into a pulicidal fluid like kerosene is effective but apt to lead to a considerable mortality Webster[119] therefore recommend the picking off of fleas and other parasites with a forceps or by hand after some suitable repellant such as spirits of camphor has been applied with a cotton swab but this tedious method is effective only when practised by a trained worker Application of DDT would be far less time consuming and fully effective but as will be noted later this insecticide is to some extent toxic for the rodents as well as deleterious to their fleas

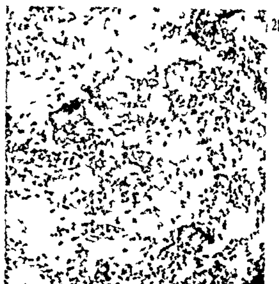
Since it is usually difficult to fulfil these conditions it is certainly preferable to use guinea pigs white mice or white rats for plague laboratory work whenever possible

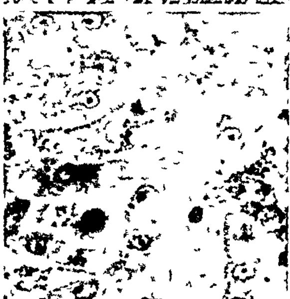
Monkeys

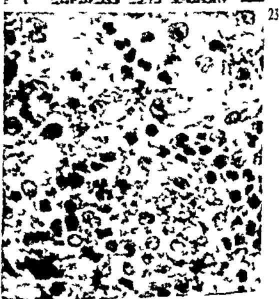
Though numerous plague experiments with monkeys belonging to different species have been undertaken for various reasons these animals have proved unsuitable for routine work with *P pestis* As has been noted before even in monkey species generally amenable to the infection marked differences in the susceptibility of individual animals have been found to exist In laboratories situated away from their natural habitats monkeys are difficult to keep while in countries like India where they are at home they may suffer from natural plague

It is interesting that in contrast to these experiences Robic[101] found some species of lemurs (locally called makis) were easily kept in the laboratory and were eminently suitable for plague research When infected subcutaneously these animals succumbed to an acute septicaemic form of the disease after three days i e before the guinea pigs and rats infected with identical doses of plague bacilli The makis could also be effectively immunized with the aid of the EV strain and were apt to contract pneumonic plague if infected intranasally









BUBONIC PLAGUE WITH SECONDARY SEPTICAEMIA IN MONKEYS

FIG 20 LYMPH NODE
P Pestis parenchyma (Giemsa)
(×225)

FIG 21 LUNG
Alveoli and blood vessels dilated numerous P pestis
(×225)

FIG 22 LIVER
Congestion of sinusoids and central cells clusters of P pestis in Kupffer s cells and blood vessels
(×450)

FIG 23 SPLEEN
Marked congestion of sinusoids and areas of hemorrhages P pestis throughout (Giemsa)
(×450)

Bats

Leger & Baury [61] found the African bat *Nyctinomus pumilus* so suscep tible to plague infection that they recommended its use as a test animal It should be noted in this connexion that the European species of the bat *Vesperugo noctula* was also found to contract the disease rapidly when inoculated subcutaneously (Gosio [43]) and that Barrera [8] could infect an Argentinian species by scarification The Japanese bat *Vespertilio abramus* on the contrary proved in the experience of Ohwada (quoted by Dieudonne & Otto [76]) rather resistant to plague infection

Birds

Domestic birds like the pigeon or the chicken being resistant to the usual methods of plague infection are of some importance for differential diagnostic purposes Kossel & Overbeck [59] for instance recommended inoculation of pigeons as an easy means of distinguishing between plague and fowl-cholera bacilli It should be noted in this connexion that although some exceptions have been recorded birds are generally resistant to infec tion with pseudotuberculosis as well as with plague bacilli (Poppe [99])

PNEUMONIC PLAGUE IN EXPERIMENTAL ANIMALS

Animals Used

As summarized by Wu Lien teh [1 4] and Pollitzer [98] earlier workers used the following animals when trying to produce pneumonic plague experimentally camels cats dogs commensal mice guinea pigs monkeys rabbits rats sisels and tarabagans (Siberian marmots)

Recent workers such as Bablet & Girard [4] Girard [3 36] Favarel [9] and Neel [83] relied mainly on guinea pigs but white mice were used as well by Herbert [49] and Meyer et al [80] the latter also experimenting with cotton rats Robic [101] and Favarel [8] found the maki fully suitable to produce primary lung infection with *P pestis*

Methods of Infection

Besides inhalation various other methods have been utilized by different workers with the aim of producing experimental infections comparable to primary pneumonic plague in man Most important among these procedures were

(a) *Intratracheal infection* originally used by Wyssokowitz & Zabo lotny [129] and later by workers such as Bablet & Girard [4] Girard [3 3] Favarel [29] and Neel [83]

(*b*) *Intranasal infection*, first employed by Batzaroff[10] and recently by Meyer and co workers[80] and—in the case of the maki —by Robic,[101]

(*c*) *Direct intrapulmonary injection* of plague bacilli as recommended by Shibayama[104] Advantage of this procedure seems to have been taken by only a few workers, e g , Wu Lien teh & Jettmar[1 6] and, recently Favarel[78] when infecting ' makis

Evaluation of Results

It has been much debated not only how easily a process identical to primary pneumonic plague in man could be produced in experimental animals but whether it was at all possible to produce such a syndrome in the latter particularly in guinea pigs and rabbits

It should be noted in this connexion that the claim of Wyssokowitz & Zabolotny[1 9] to have produced primary pneumonic plague in anaesthetized monkeys was not confirmed by Nattan Larrier or by Broquet (quoted by Bablet & Girard[4]) who had access to the specimens of the two Russian workers

General agreement exists that Batzaroff[10] had been successful in pro ducing this form of plague in guinea pigs rabbits and monkeys by intra nasal infection and had occasionally obtained the same result in rats and mice as well However Bandi,[6] repeating such tests with guinea pigs and also performing inhalation experiments with this rodent species and with rats, considered the resulting lung changes to be secondary in nature plague *infection reaching the lungs through the lymphatic channels*

Strong & Teague[111] who were also unable to produce primary pneu monic plague in guinea pigs through inhalation, ascribed this failure to the shallow type of respiration in these animals or to the small size of their larynx Shibayama[104] incriminated the thick cluster of hair present in the nostrils of this species and maintained that the only method of causing primary pneumonic plague in guinea pigs was to inject the infective material directly into the lungs

The evidence regarding the rabbit was also contradictory Martini asserting, in contrast to other observers, that septicaemic and not *pneu* monic infection resulted when plague bacilli were administered to these animals by *inhalation (Wu Lien teh*[1 1]) Attention was also *drawn to* the fact that pneumonia like processes could be produced in test animals through the action of plague toxins Signorelli,[106] for instance *having* been able to produce such a process by injecting Lustig s nucleoprotein into the lungs of dogs

However without denying that some investigators were unable to produce primary lung involvement in experimental animals it would be altogether wrong to consider their negative results as generally valid or even to assume, as some observers did, that pneumonic *plague in animals*

as well as in man was invariably secondary in nature the infection entering through superficial mucous membranes instead of directly reaching the deeper respiratory tract For irrefutable proof of the existence of primary pneumonic plague in adequately infected animals and also—as will be shown later—in man has been brought forward by several workers

The following findings recorded in this connexion deserve attention here

Strong & Teague[111] found that monkeys infected by inhalation practically always developed primary lung involvement showing only slight and obviously secondary changes in the faucial and cervical tissues Tonsillar infection on the other hand resulted in plague septicaemia with or without involvement of the cervical lymph nodes but never led to pneumonic changes

Wu Lien teh & Jettmar[116] performing inhalation experiments with tarabagans and sisels definitely showed through histological examinations that the primary focus of infection was invariably situated in the deep portion of the respiratory tract

Nattan Larrier & Richard[8] insisted upon the profound difference of the histological findings in experimental animals with primary and secondary pneumonic plague respectively In the former a bacillary alveolitis was in the foreground while the latter showed an abundance of the bacilli in the lymphatics of the lungs

Bablet and Girard[4] also relying on careful histological examinations *obtained full proof that in intratracheally infected guinea pigs* bacteraemia was secondary to changes in the deep respiratory tract

Recently Meyer and colleagues[80] infecting white mice guinea pigs and cotton rats intranasally and Neel[83] practising intratracheal infection of *guinea pigs also definitely established that in their animals bacteraemia* was secondary to the process developing in the lungs

Evolution of Primary Pneumonic Plague in Animals

Describing the evolution of the morbid process in guinea pigs infected with plague by the intratracheal route Bablet & Girard[4] distinguished between three successive phases

(*a*) A broncholymphatic stage characterized not only by an inflammatory reaction involving the mucosa and adjoining lymph spaces of the trachea and main bronchi but—as early as eight hours after infection—also by circumscribed alveolar lesions (bronchio alveolite pesteuse) round some intralobular bronchioli

(*b*) A pneumonic phase becoming manifest 24 30 hours after infection and characterized by numerous lobular foci which were often situated around bronchioli In due course extension and multiplication of these foci led to the formation of plague abscesses rich in plague bacilli and leucocytes which showed a tendency to break through into the bronchi

(c) A short septicaemic stage demonstrable not earlier than 60 hours after infection through blood cultures At the same time the pneumonic foci became confluent and plague bacilli abounded in the lumen of the bronchi and the trachea

Examining mice and guinea pigs killed at intervals of 6 to 12 hours after nasal instillation with plague material, Meyer et al [80] noted the evolution of a process similar to that mentioned above These workers described *the gross findings in mice which had succumbed to the infection (usually* after 72 96 hours) in the following terms

The gross anatomical lesions in the mice vary slightly In rapidly fatal infections a small amount of fluid is present in the chest cavities all the lobes of the lung are dark red in a state of diffuse exudative hemorrhagic edema or there is lobular red consolidation in the left or right anterior or diaphragmatic lobe Edematous fluid oozes from the trachea and occasionally from the nostrils Parenchymatous changes in the liver and kidneys as well as fatty hemorrhages in the adrenal complete the picture of a toxemia

The macroscopic features in intranasally plague infected guinea pigs which as a rule were not visibly ill for the first two days but suddenly died between the 3rd and 5th day after infection were thus characterized by Meyer and co workers

Since in contrast to mice guinea pigs are much more resistant to plague toxin the gross lesions aside from a generalized subcutaneous capillary injection show little or no fluid in the pleural cavities The lungs as a whole are voluminous and portions of the apical or principal lobes are in different stages of red and gray hepatization The pleural covering of these areas is rarely covered with fibrin The bronchi and trachea are injected and covered with blood streaked mucus Bronchial and mediastinal lymph nodes are always enlarged edematous and occasionally studded with early necroses the regional lymph nodes of the neck and submaxillary space as a rule are not involved and the spleen is not enlarged The infection is definitely confined to the respiratory tract

NATURAL PLAGUE IN ANIMALS

Commensal Rats

For different reasons the autopsy findings in rats which had contracted plague under natural conditions may show considerable variations

Even during epizootics much depends on the way in which the material for dissection is collected Examining exclusively the animals found dead, one may except to come across a high percentage of specimens showing in addition to frankly positive bacteriological findings marked gross lesions Even then, however, an appreciable number of rats may be found which having apparently succumbed rapidly to an overwhelming infection show little or even no macroscopic evidence of this although their organs teem with plague bacilli

The percentage of rats showing marked gross signs of plague decreases in relation to the proportion among the dead rats examined of animals killed by the population of the plague threatened area Nevertheless, during epizootics one may hope to find even among the killed rats a number of specimens showing marked macroscopic evidence of infection—apparently animals which had been less able than their healthy mates to evade their pursuers

Results of macroscopic examination are, however, apt to be rather disappointing if trapping methods are used to obtain rats for dissection Even during epizootics though carcasses showing incipient plague or occasionally displaying signs of having recovered from it may be encountered animals with fully developed macroscopic features will be few and far between because as aptly pointed out by Hundley & Nasi [50] rats suffering from plague generally prefer to stay in their nests It is true that delirium and/or the desire for fresh air may ultimately drive them out but although prone to be killed while staggering about such moribund animals will not be attracted by the baits offered in the traps

As shown by Shih & Pollitzer [10] who had opportunities to examine many hundreds of rats which included animals found dead and animals killed both during and between epizootics the frequency of specimens showing marked pathognomonic signs at autopsy was definitely related to the stages of the epizootics The number of such specimens started to increase at the onset of the plague seasons reached a maximum at their peak and then gradually declined to become minimal during the off seasons The frequency of grossly affected rats and correspondingly the number of animals showing abundant bacteriological evidence of the infection were thus apt to serve as a yard stick for assessing the seriousness of the situation this naturally also holds true when the number of animals available for examination is small

It further stands to reason that the frequency of rats showing marked macroscopic lesions will vary according to the type of plague prevailing in the area in question Thus it was claimed [53] that in Brazil rat plague was generally manifest in an unusually mild and atypical form In regions where the rat population is becoming resistant to the infection results of macroscopic examination are also apt to be less clear cut than in areas with a fully susceptible rodent population

Autopsy findings

While the description of the postmortem findings in plague affected rats is based mainly on the classical studies published by the Plague Research Commission in India in 1907 [91] further contributions to the knowledge of this subject have been made by other workers recently for instance by Prado [100] Macchiavello [70] [71] de Smidt [106] de Moura & Remião [81] and Hundley & Nasi [0] The results of these investigations may thus be summarized

Rigor mortis As pointed out by the Plague Research Commission rigor mortis, often present in plague affected rats and sometimes persisting even after putrefaction has set in, is somewhat characteristic, the limbs projecting stiffly from the body in a distinctive manner

Subcutaneous tissues One of the common features of rat plague is subcutaneous congestion, which may manifest itself by a reddish hue of the skin before the rats are dissected, being specially visible on the plantar surface of the hind feet However, as stressed by the Commission, this sign may be absent in plague affected rats and present in animals succumbing for other reasons Among subsequent workers Paisley[89] and Gomila[41] laid great stress upon pink discoloration not only of the feet, but often of the abdomen and thorax also, Gomila finding 'pink feet' in 75% of his plague positive rats

Another point noted by the Commission and other observers was a peculiar purplish red appearance of the thoracic and abdominal muscles, due to the presence of congested vessels In conjunction with the reddish pink colour of the subcutaneous tissues this appearance was considered as strongly suggestive of plague infection

The Plague Research Commission found subcutaneous haemorrhages to be quite frequent in plague infected rats and emphasized at the same time, the absence of this feature in animals not so infected The haemorrhages were most often situated in the submaxillary region even if no bubo was present in the neck or elsewhere, next in frequency came the region of the flank, while in young or medium sized rats the haemorrhages were apt to be widespread

In the experience of the Plague Research Commission a general subcutaneous oedema, which forms a characteristic feature in experimentally infected guinea pigs, was rare in naturally plague affected rats

Lymph nodes In describing the changes in the lymph nodes of plague affected rats, the Commission drew a sharp distinction between a process of general involvement and primary buboes Enlargement and congestion of the lymph nodes in general was met with in septicaemic plague and also as the result of secondary invasion through the blood stream in rats showing a primary bubo Infection could also spread from the original bubo through the lymphatics to neighbouring lymph nodes thus leading to the formation of 'primary buboes of the second order'

The morbid appearances in primary plague buboes depended upon the stage of the infection At first the affected lymph nodes showed only some enlargement and congestion with haemorrhagic points on the cut section Later, signs of necrosis became manifest, affecting either the medullary portion (or even merely part of it) or, in advanced cases the whole substance of the nodes involved Ultimately disintegration took place with the formation of usually rather dry purulent material

In well developed infections the tissues round the primary buboes were as a rule affected often showing infiltration as well as marked congestion and haemorrhages and sometimes a localized subcutaneous occasionally gelatinous oedema

While as a rule the prominent swelling caused by the relatively large primary buboes was apt to attract attention their existence was sometimes suggested merely by slight asymmetry and confined to signs of some necrosis on the cut section The Plague Research Commission therefore recommended the incision of all lymph nodes, even if they were not outwardly changed and did not exhibit the peculiar hard consistency often noted in plague buboes Axillary buboes were particularly liable to be overlooked as they might be small flattened and lying parallel to the inner surface of the upper extremity under the lateral insertion of the pectoral muscle It was therefore necessary to cut through this muscle to reveal the axillary cavity

Analysing the results of 4 000 autopsies of naturally infected rats the Plague Research Commission found involvement of the lymph nodes in 84 72% (buboes in single situation in 73 05%) and absence of buboes in 15 25% The distribution of the buboes in single situation was as follows

Neck	75 0
Axilla	15 1
Groin	6 1
Pelvis	3 8

The bubo distribution recorded by other workers was sometimes not in full accord with that shown above but it should be kept in mind that as a rule they based their conclusions on much smaller samples than those available to the Plague Research Commission In the fairly large amount of material examined by Pollitzer in China cervical buboes were the most frequent

Liver Although the liver of rats yielding ample bacteriological evidence of infection with *P pestis* may show at autopsy only slight and uncharacteristic changes if indeed any plague infected rats often display lesions of the organ which may be considered pathognomic for this infection As established by the Plague Research Commission these characteristic lesions were twofold in nature

On the one hand appearances were frequently present which macroscopically seemed due to fatty changes but as revealed by the microscope were actually caused by necrosis In the early stages of this process a characteristic mottled appearance was produced by the contrast between the yellowish aspect of the parts with fatty changes and the reddish colour of congested areas In advanced cases the liver had a pink tinge and no signs of division into lobules were seen The organ appeared as

though modelled in wax with the outer surface peculiarly dome like and the edges sharply defined, it was no longer elastic but rather friable Although these changes appeared to be pathognomic in fresh carcasses a similar condition of the liver was sometimes found in putrid rats which had not succumbed to plague

Another change found in 58% of the 4 000 rats occasionally side by side with fatty appearances, consisted of the occurrence of necrotic foci scattered over the surface and throughout the substance of the liver (" granular liver) Typically these nodules were of pin point size and if superficially situated, not bulging However in some cases—apparently of longer standing—the granules were coarser, those on the surface were raised above the liver tissue and, although good cultures were obtained, only a few bacilli could be seen in microscopic preparations from the organ

Spleen In well marked cases of rat plague the spleen was enlarged and firm with a moulded appearance so that it was found lying over the stomach instead of being out of sight like a soft normal spleen It must be noted however, that a similar enlargement of the spleen may not infrequently, be met with in rats not suffering from plague More characteristic was the presence of smaller or even confluent larger nodules but, according to the Plague Research Commission, such nodules were found in only 4 5% of the plague rats, being thus considerably less common than in the liver

Kidneys and suprarenals The kidneys were often congested and not infrequently showed subcapsular haemorrhages 'Fatty changes were frequent but the presence of plague nodules was exceptional The suprarenals were often enlarged and congested

Stomach and intestines In the experience of the Plague Research Commission, no characteristic changes were found in the stomach and intestines of plague infected rats, subserous haemorrhages occasionally seen on the stomach, were rarely present in the intestines

Although most workers confirmed these observations, a few found marked changes in the gastro intestinal tract Paisley [89] and Connal et al [19] noted in some of the plague infected rats in Lagos, Nigeria, subserous haemorrhages and bloody effusion in the small intestines Mesenteric buboes were present in such cases Prado [100] in Brazil found plague infected rats with enlarged and haemorrhagic mesenteric lymph nodes and also noted sometimes the presence of the same changes or of caseous pus in retroperitoneal lymph nodes of the lumbar region In rare instances he observed in the intestines elliptic white patches corresponding in size to Peyer's patches which were in a process of cicatrization or seemed to have become cicatrized It was assumed that the rats showing these intestinal lesions had contracted infection by devouring plague carcasses

Pleurae and lungs Haemorrhages were fairly often noted in the pulmonary pleurae and lungs but apparently never in the parietal layer of the pleurae

Pleural effusion was a fairly frequent sign in plague infected rats it was met with in over 70% of the animals found to be plague positive by the Plague Research Commission Macalister & Brooks,[69] who had the opportunity of comparing the findings in a small number of plague affected rats with those in large numbers of plague free animals laid stress upon the clear appearance of the fluid because in the case of trypanosomiasis a blood stained exudate was found However as noted by the Plague Research Commission and confirmed by other workers a slightly or moderately blood stained effusion may be found in plague infected rats as well Moreover though trypanosomiasis is fairly frequent among the rats in China, Pollitzer failed to observe pleural effusions in them unless they were infected with plague

While it may be asserted that until recently the presence of an appreciable or abundant amount of clear pleural fluid proved the existence of plague in the rats concerned it must be noted that nowadays a pleural effusion of the same character may be encountered in rats which have been poisoned with α naphthylthiourea (ANTU)

Though a certain degree of congestion and/or oedema might be present in the lungs of plague affected rats more marked or characteristic changes were as a rule absent Nodules were rare being present in only 2 5% of the Plague Research Commission series and primary pneumonic changes quite exceptional

Pericardium and heart Pericardial effusion was fairly frequent in plague affected rats Epicardial haemorrhages were occasionally present and the superficial vessels were often congested The walls of the heart were relaxed the right cavities usually engorged with blood and the left empty

" Chronic plague in rats

Kolle & Martini[58] seem to have been the first observers to note the existence of a chronic form of plague in rats which had been experimentally infected months before The animals in question showed caseation of the bronchial lymph nodes induration of the lungs and encapsulated foci in the submaxillary lymph nodes with positive bacteriological findings Similar results were afterwards obtained by Hata (quoted by Pollitzer[93]) in rats and guinea pigs treated first with small doses of weakly virulent *P pestis* and then with fully virulent material Thus an experimental basis seemed to have been furnished for the belief of early workers such as Simond[107] and Gotschlich[4] that chronic rat plague might be the means for perpetuating the infection during the off seasons between epizootics

A profound study of this problem was carried out by the Plague Research Commission in India (1906 12 [93 97]) This work, supplemented by the exhaustive histological studies of Ledingham,[63] showed the presence of different categories of apparently " chronic ' lesions in naturally plague infected rats As summarized by Petrie,[91] a distinction could be made between " *peripheral lesions, consisting of encapsulated abscesses* corresponding in size and relative frequency to the primary plague buboes, and 'visceral lesions usually found in the spleen and consisting either of thick walled abscesses, often attached by adhesions to the surrounding serous membranes or of necrotic areas in the spleen substance Plague bacilli were often *absent from such lesions if present, they were not abundant, though usually* virulent In other instances in which bacteriological findings were invariably negative, adhesions of the spleen to the surrounding structures, scars on this organ or fibrous thickening of its capsule were found singly or in varying combinations

The Commission was careful to point out that, except in cases with positive bacteriological findings, it was not possible to obtain direct proof that such lesions were caused by plague In fact similar appearances were found in Madras and Poona rats which had definitely not been exposed to plague infection It could be shown, however, that

(1) Similar lesions were present in certain experimentally infected rats Specially noteworthy was their presence in 11% of rats from different parts of India which had survived inoculation with small doses of plague bacilli and had been killed three weeks afterwards, the largest proportions of such lesions were found among the survivors of the most susceptible group

(2) The lesions found in rats exposed to natural infection were of an identical character, regardless of whether or not plague bacilli could be detected in them, in fact sometimes both foci, yielding positive and negative bacteriological findings, were present in one and the same animal

(3) Rats with such lesions increased in number during and after plague epizootics

The Commission found no evidence whatsoever to show that lesions still containing plague bacilli had been the source from which an acute infection had been re established in a particular rat On the contrary, these lesions seemed to represent successive stages in the natural process of recovery from acute plague infection For this reason the Commission proposed for them the designations resolving and resolved plague in preference to the term chronic plague, in order to avoid the erroneous impression that rats with such lesions were instrumental in perpetuating the infection

While these views have been accepted by most investigators a few expressed divergent opinions

Swellengrebel & Otten[11] described a mitigated form of plague in a naturally infected rat and were able to produce identical lesions in partly immune rats as well as in guinea pigs inoculated with material of low virulence or infected cutaneously with minimal doses of virulent plague bacilli The changes observed in such animals consisted of multiple small axillary or inguinal buboes haemorrhages in all organs and sometimes exudates in the serous cavities Plague bacilli could not be directly demonstrated and infection of susceptible animals was apt to produce at first a similar process However further animal passage finally led to typical plague Swellengrebel & Otten ascribed some importance to this mitigated form of plague believing that the process might pass, under natural conditions to the acute form

Bordas et al[16] and Williams & Kemmerer[1] drew attention to rats, showing only trivial or no lesions and found especially at the end of the epizootics from which plague cultures of supposedly low virulence could be isolated Tanon (quoted by Petrie[91]) expressed the idea that by continued passage from rat to rat such strains might regain virulence and lead to a severe epizootic

Williams[11] besides reporting on two naturally infected rats which had suffered apparently from resolving plague yet showed bacteraemia experimented with 2 278 *R norvegicus* killing the animals surviving cutaneous or subcutaneous plague infection at different intervals Among the rats showing signs suggestive of resolving plague one killed 21 days and four killed 30 days after inoculation showed plague bacilli in the heart blood immediately after death Williams suggested that these experiments completed the proof of a perpetuation of the infection through plague strains of low virulence as suggested by Tanon, and formed the tentative hypothesis that plague produced among the rats a certain proportion of carriers in which bacteraemia might appear at times with consequent opportunities for infection of fleas and a spread of the disease to other rats

Recently Savino et al[10] finding that one rat out of almost 120 000 dissected showed features of resolving plague and yet yielded positive results in cultural and animal tests expressed the opinion that rats suffering from this form of the disease might be the means of carrying over the infection

Even apart from the fact that the claim made by some of these authors to have found in their rats plague strains of a low virulence has not been supported by sufficiently exact tests the evidence recorded and the theories put forward by them cannot be considered as convincing

Nobody will deny that in a small proportion of rats suffering apparently from resolving plague bacteraemia may still persist or in other words that no sharp dividing line can be drawn between instances of acute or subacute and those of resolving rat plague—a fact stressed by the Plague Research Commission That bacteraemia could persist in such animals

for more prolonged periods or that it could reappear in the course of the resolving process has, however, never been proved As Petrie[91] maintained with much reason

> such an event is unlikely to happen because during the process of recovery from an acute attack rats presumably acquire a considerable immunity and moreover *there is no experimental evidence either in plague or in any other similar infection* to support the idea that a chronic focus is able to kindle a fresh septicaemia

A few workers have drawn attention to the occurrence in commensal rats of an 'inapparent' form of plague in which macroscopic signs of the infection were absent and direct bacteriological examination yielded negative results, yet animal experiments proved positive However, it was not claimed that such animals played a role in the perpetuation of the infection On the contrary, Peryassu[90] denied that his carrier rats were responsible for plague outbreaks and Devignat,[3 24] who established the presence of the infection in apparently healthy rats through animal experiments with their pooled bone marrows noted that human plague was absent from the localities where such rodents had been found

Commensal Mice

As a rule, naturally plague infected commensal mice show no characteristic postmortem changes General signs of infection, particularly swelling of the spleen, may be present but these are also met with in animals which do not succumb to plague However, Pollitzer not infrequently noted in the commensal mice he dissected, a brick red discoloration of the liver, sometimes associated with a mottled appearance due to the presence of yellowish areolas Almost invariably mice showing such liver changes were found to have succumbed to acute plague

Wild Rodents

Although as will be discussed later numerous naturally plague infected wild rodent species have been found few detailed descriptions of the morbid appearances met with in such animals have been recorded The available information may be summarized as follows

Siberian marmots (tarabagans)

As stated by Wu Lien teh[1 1] 15 Siberian marmots (*Marmota bobak*) found naturally plague infected up to 1924 could be classified thus

bubonic plague	11
? pneumonic plague	1
? septicaemic plague	1
? residual plague	2

Not all these animals were in a condition suitable for an exhaustive examination As far as could be established cervical buboes preponderated

being found in 9 of the 11 animals showing features of bubonic plague together with axillary buboes in two cases.

The spleen was usually more or less swollen and softer, sometimes showing small greyish nodules. The liver showed, as a rule, signs of congestion and occasionally also yellowish white nodules and/or haemorrhages. Secondary lung involvement was frequent, as confirmed by the histological studies of Wu Lien-teh and Lin Chia Swee.[117]

Rodents of south-east Russia

Sisels (susliks). In naturally infected susliks (*Citellus pygmaeus*) all forms of plague—bubonic, pneumonic and intestinal—have been diagnosed according to Nikanoroff;[85] transitions among these types have been frequently met with. Susliks displaying no marked macroscopic lesions yet yielding virulent plague cultures were also often seen.

Among the animals with well-marked features of plague the bubonic type preponderated, Suvoroff (quoted by Nikanoroff[85]) for instance finding buboes in 44 (84.6%) out of 52 naturally infected susliks. The inguinal lymph nodes were most frequently involved.

Signs of residual plague were often manifest. Thus Nikanoroff[84] investigating a district where plague had been active the year before found lesions of this type in the lymph nodes of the susliks, particularly in those in the groin.

Gerbils. Studying the morbid anatomy and histology of experimentally infected gerbils (*Meriones meridianus*) Lobanov & Fedorov[66] found evidence for the existence of two different types of plague with a distinct seasonal incidence. In spring and early summer (April to July) the character of the disease was fairly benign with a tendency towards the formation of localized abscesses which became encapsulated and absorbed and were finally replaced by scar tissue. In late summer and autumn (August to October) on the contrary an acute type of generalized plague was prevalent.

South African rodents

According to Pirie[9] experimentally infected wild rodents involved in the plague outbreaks in South Africa as a rule showed no marked gross changes, the most constant signs being slight and general subcutaneous congestion and more marked engorgement of the vessels behind the sternum and front of the chest wall. Since the animals had been inoculated percutaneously on the inside of the left hind leg, inguinal buboes were usually present but were not conspicuous in animals which had survived less than five or six days. In those succumbing after three or four days at most a slight, soft, sometimes haemorrhagic swelling of the inguinal lymph nodes was apparent. The spleen was usually somewhat enlarged and presented a duller and more opaque appearance than in normal

animals In the lungs small patches of incipient pneumonic plague were frequent

It was not possible to say definitely whether similar alterations were present in naturally infected animals because only small numbers of these could be examined The available evidence suggested that in nature also an acutely septicaemic type of plague prevailed Chronic lesions were never met with in the multimammate mice and were extremely rare in the gerbils

Californian rodents

As summarized by Wu Lien teh[121] McCoy found in a representative series of 246 naturally infected Californian ground squirrels (*Citellus beecheyi*) acute plague in 60 (24 4%) all with buboes, subacute plague in 152 (61 8%) 73 7% with buboes, and residual buboes in 34 (13 8%) Inguinal buboes were most frequent followed by involvement of the cervical and lastly, of the axillary lymph nodes Primary lung involvement was exceptional, but secondary lung lesions, consisting of the presence of nodules or areas of grey hepatization were common

Again dealing with the pathology of plague in Californian wild rodents Meyer[6] stressed the frequency of lesions in the lymph nodes as well as a striking tendency towards ' latent infections without the presence of gross features Meyer also stated that secondary, or even primary, pulmonary localizations were much more common in these animals than in rats Involvement of the lymph nodes adjacent to the gastro intestinal tract—apparently due to gastro intestinal infection as the result of cannibalism—was according to Meyer s observations by no means infrequent

The diversity of the morbid lesions met with in the plague affected rodents of California was, in Meyer s opinion connected with a varying susceptibility of the animals during and after hibernation Subacute and resolving plague were the prevailing types during winter[77]

In contrast to the opinion of Kellogg[64] Meyer[7] found no evidence that the plague strains isolated either from Californian wild rodents or during the pneumonic epidemics at Oakland and Los Angeles, from human sources possessed any peculiar pneumotropic properties

Argentinian rodents

As maintained by Alvarado & Barrera[3] (see also Barrera[7]), a sub acute type of plague without marked gross lesions was prevalent among the various species of *Microcavia Graomys* and *Galea* involved in the plague outbreaks in Argentina Instances of inapparent infection where animal experiments alone yielded a positive result were met with (Outes[88]) An atypical form of plague was described by Barrera[8] in which hypertrophy congestion and haemorrhages of the testicles were present

"Inapparent" Plague in Wild Rodents

A form of plague occurring in naturally infected wild rodents and characterized by the absence of gross findings negative results of direct bacteriological examination yet positive experimental results with the organs of the animals in question has been described by workers in the Union of South Africa south east Russia, and California (Meyer et al [79]) and recently also by Outes [89] in Argentina and by Baltazard & Mofidi [5] in Persia The Russian observers of this type of the disease (variously designated as plague with invisible lesions latent or inapparent plague) advanced the hypothesis that if the rodents involved became subjected to adverse environmental influences they might develop bacter aemia and being thus apt to infect their fleas might then be instrumental in perpetuating the disease

Thus far no proof for this assumption has been brought forward In fact as Baltazard & Mofidi [5] seem to suggest it is by no means certain that inapparent plague is a type sui generis because the animals in question might have been either in an initial stage of the infection or in the process of recovery from it

As will be discussed in chapter 6 definite evidence exists that in hibernating wild rodents the infection may remain localized throughout the winter sleep at the portal of entry and/or in the regional lymph nodes to become generalized after the animals have awakened in spring In Pollitzer s opinion it is so far not justifiable to identify this latent type of plague with the inapparent form of the infection claimed to exist in non hibernating rodents

HUMAN PLAGUE

A classification of human plague has been attempted in various ways the point of difference being usually the position accorded to the septi caemic type As commonly defined this is a form of the disease where as in bubonic plague the causative organisms enter through the skin (or rarely through superficial mucous membranes) but where owing to an overwhelming infection or to a lowered resistance of the host the regional lymph nodes are as it were, overrun so that an immediate invasion of the blood stream takes place

It must be noted however that an apparently primary septicaemia may be actually due to a secondary invasion of the blood stream either because buboes developing in deep seated lymph nodes may escape clinical detection (the hidden buboes of Wright [123]) or because as the result of a rapidly progressing infection the reactions taking place in super

ficial regional lymph nodes may remain so inconspicuous as to be quite overshadowed by the most serious general condition of the patients It is of great interest to note in this connexion the contention of Girard[34] that what is considered as primary septicaemic plague is really a form of the disease with inconspicuous buboes

It must be further emphasized that, in contrast to the commonly held beliefs not only an entry of the infection through the skin but also a direct invasion of the deep parts of the respiratory tract may produce a rap[...] generalized type of the disease without conspicuous local reacti[...] Girard[38, 39] recently spoke in this connexion of a "clinically septicaem[...] form of pneumonic plague

For these reasons it seems preferable to follow the lead of Crowell who distinguished between two main types of human plague only—th[...] primary bubonic and the primary pneumonic (or more correctly pulmonary) form As will be discussed in chapter 8 (p 439), recently the terms zootic" and demic have been proposed to designate these two main forms of plague

Pathology of Bubonic Plague

Route of infection

The skin forms the usual portal of entry in bubonic plague, infection being produced in the overwhelming majority of cases through the bite of infected rodent fleas rarely through that of other blood sucking insects or directly through contact with infected rodents human victims or contaminated inanimate objects Another rare group of cases are those where infection entered not through the skin but through superficial mucous membranes, such as tonsils and conjunctiva

Bacteraemia

The statements of different observers as to the frequency of bacteraemia in bubonic plague vary considerably but no doubt can exist that these discrepancies were due in the first place to the different amounts of blood used for examination In particular as shown by the Plague Research Commission[93, 97] microscopical examination of blood smears alone was unreliable As established by the Commission severe bacteraemia might be present at a comparatively early stage of the disease but as a rule its degree was in an inverse relation to the length of time between examination and death of the sufferers While bacteraemia was thus usually progressive in character diminishing , irregular , and fluctuating types were met with Patients showing bacteraemia might recover

Later observations confirmed that bacteraemia was by no means always restricted to fatal cases of bubonic plague Thus Teissier et al[111] came to the conclusion that even in mild cases of bubonic plague an early bacillaemia was nearly always demonstrable and that its presence in the

later stages alone rendered the prognosis unfavourable Ohoto [86] obtained positive results in 72 2% of his material bacteriemia disappeared in mild cases as early as the second day and not later than the tenth while persisting in severe cases Kirschner [5] using preliminary cultivation in bile found bacteriemia in 212 out of 237 definite plague cases Bonebakker,[1] who also used bile media in 85% of his bubonic plague patients Schoebl [103] stressed the diagnostic value of blood cultures in plague Gonzaga [4] coming to the same conclusion stated that bacteriemia occurred early in ordinary bubonic plague when diagnostic advantage might be taken of its presence

The results obtained by Jawetz & Meyer [51] when infecting guinea pigs mice or rats intracutaneously or subcutaneously with relatively small non toxic doses of *P pestis* serve as a corollary for the above recorded observations As stated by these two workers their findings

> tend to confirm Teissier s claim because an invasion of the blood stream always follows the early lymphatic spread in experimental animals after the local resistance has been overcome The initial bacteraemia may last for only a very short time due to the effective filtering action of the liver and spleen The filters are overcome and bacteria appear again in the circulating blood only after the massive multiplication of organisms Active multiplication of *P pestis* in the blood stream is probably always terminal "

Action of endotoxin

While as discussed above even in apparently uncomplicated cases of bubonic plague a passing bacteraemia seems the rule and this incessantly progresses in cases ending fatally the important role played by the endotoxin liberated in the course of the disease must duly be taken into account

As stated by Dieudonne & Otto [26] the marked prostration and the disturbances of the sensorium conspicuous in severe plague cases are no doubt due to an action of the endotoxin which according to Girard & Sandor [40] and Girard,[37] exhibits a marked neurotropism

The widespread haemorrhages and degenerative changes in the internal organs conspicuous in plague victims are likewise the result of a generalized absorption of the endotoxin while the foci of necrosis associated with bacillary emboli in the organs are ascribed by Petri [81] to local liberation of the endotoxin

It is interesting to note in this connexion that the German Plague Commission [3] found in three foetus from plague infected mothers haemorrhages and parenchymatous degeneration of the organs but no bacilli which in contrast to the toxin were apparently unable to pass through the placenta However some cases of true intra uterine infection with *P pestis* have been noted since (Wu Lien teh et al [1, 5] Haffkine quoted by Wu Lien teh [1, 4] Bikov [1])

Of special importance is the fact that the high concentration of toxin within and in the vicinity of the primary buboes leads to haemorrhagic

infiltration of the walls of the neighbouring veins (first noted by Childe[19]) and consequently to the passage of the bacilli into the blood stream

Morbid anatomy

External appearances The general appearances noted in plague victims naturally depend a great deal upon the time that has elapsed after death and the temperature prevailing in the interval Generally it may be maintained that rigor mortis sets in quickly, becomes marked and lasts for a long time On the other hand, signs of decomposition in the dead bodies do not develop rapidly except in cases of mixed infection

It is striking that, although in the overwhelming majority of cases the skin forms the portal of entry for bubonic infection, as a rule no reaction is noticeable at that site or in the lymph vessels leading to the primary buboes which usually form the first manifestation of the process Sometimes, however a primary plague pustule or a carbuncle may be present at the site of infection, and inflamed lymph vessels may be seen leading to the bubo

Secondary skin manifestations such as pustules and carbuncles due to an invasion of the skin through the blood stream, are likewise rare in modern plague outbreaks though they seem to have been more conspicuous in some epidemics than in others Skin haemorrhages, usually of a petechial character, situated either in the vicinity of the primary bubo or in other skin regions may not infrequently be detected if properly looked for Franca[30] found them in Oporto Portugal 46 times in 110 autopsies and also noted carbuncles 11 times (7 of a primary nature), pustules 6 times and once a pemphigus like skin affection

Lymph nodes The classical studies of Albrecht & Ghon[1] have led to a distinction between

(*a*) primary buboes of the first order, developing in the lymph nodes draining the site of infection and as a rule, representing the local manifestation of the morbid process

(*b*) primary buboes of the secondary order i e, lymph nodes situated near to as a rule proximally from, the originally invaded lymph nodes or tissues and receiving infection through the lymph stream

(*c*) secondary buboes infected by way of the blood stream and consequently apt to be present in all types of plague where a septicaemia develops

The primary buboes of the first order are characterized in typical cases by a marked reaction in the surrounding tissues as well as by a process of haemorrhagic inflammation and coagulation necrosis in the lymph nodes themselves Many gradations of these morbid processes are met with

One or several lymph nodes may be affected in the latter case the individual nodes are often matted together and with the surrounding tissues so that considerable swellings are produced

All degrees of inflammation may be present in buboes according to the intensity of the process and the length of illness varying from simple lardaceous or medullary swelling to gelatinous infiltration and bloody infarction and from softening and liquefaction to pus formation and complete necrosis (Dieudonne & Otto [6]) The degree and extent of the reaction round the glands may also vary considerably though usually marked oedema and infiltration are present which may involve in addition to the surrounding connective and adipose tissue other structures such as veins (see above) fasciae and muscles

The primary buboes of the secondary order may show similar changes to those described above but as a rule the reaction in the surrounding tissues is less marked in degree and extent

The secondary buboes are usually smaller in size than the primary ones with the possible exception of some oedema the process is limited to the lymph nodes themselves which may show congestion medullary swelling and discrete haemorrhages

As will be gathered from the above descriptions inconspicuous primary buboes may show appearances microscopically indistinguishable from those exhibited by secondarily invaded lymph nodes

Mouth and fauces Generally speaking no characteristic changes of the oral and faucial mucosa are found in plague However some congestion and also signs of cyanosis may be present while the papillae at the base of the tongue and the pharyngeal follicles may be swollen The tonsils as a rule show corresponding changes but sometimes they are the seat of marked inflammation and ulceration with membrane formation

Though processes of this kind met with in plague victims need not necessarily be of a specific nature no doubt exists that *P pestis* may produce such lesions However the tonsillar involvement is by no means always of a primary nature often the infection entering in the usual manner through the skin leads first to the formation of cervical buboes from which the process secondarily spreads to the tonsils (de Sousa [109] Crowell) Martinez Vinueza [73] distinguished accordingly between primary and secondary anginose plague

Respiratory system The lungs may be invaded in bubonic plague by different routes In cases where the tonsils are affected a pneumonic process may be produced by aspiration Albrecht & Ghon [1, 2] for instance saw two such cases in a series of 44 autopsies

It should be noted however that tonsillar plague by no means invariably leads to such secondary localization of the infection in the lungs Thus de Sousa [109] stated that faucial involvement may remain localized

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The secondary buboes are usually smaller in size than the primary ones; with the possible exception of some oedema, the process is limited to the lymph nodes themselves, which may show congestion, medullary swelling, and discrete haemorrhages.

As will be gathered from the above descriptions, inconspicuous primary buboes may show appearances microscopically indistinguishable from those exhibited by secondarily invaded lymph nodes.

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Though processes of this kind met with in plague victims need not necessarily be of a specific nature, no doubt exists that *P. pestis* may produce such lesions. However, the tonsillar involvement is by no means always of a primary nature, often the infection, entering in the usual manner through the skin, leads first to the formation of cervical buboes from which the process secondarily spreads to the tonsils (de Sousa[109], Crowell). Martinez Vinueza[3] distinguished accordingly between primary and secondary anginose plague.

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It should be noted, however, that tonsillar plague by no means invariably leads to such secondary localization of the infection in the lungs. Thus de Sousa[109] stated that faucial involvement may remain localized

and clear up, the sputum never containing numerous plague bacilli. Crowell found that in 11 cases with pharyngeal lesions, lung involvement was present seven times.

A number of observers laid great stress upon a direct spread of plague infection from cervical or axillary buboes to the lungs (see Wu Lien-teh[121]). Gaud & Jorge[31] maintained in this connexion that, among the five groups of axillary lymph nodes, the thoracic or 'subpectoral' one was most often involved in plague and, as these lymph nodes were in communication with the intrathoracic lymphatics, a direct invasion of the lungs was likely.

Though due attention should certainly be paid to a direct invasion of the lungs from cervical or axillary buboes, its importance should not be exaggerated. Secondary lung involvement is by no means restricted to cases with axillary or cervical buboes. On the other hand, localization of the infection in the axillary or cervical lymph nodes is by no means invariably followed by secondary pneumonia.

Thus Crowell, who paid special attention to this point, found pneumonic changes in only six out of ten cases with cervical buboes and concluded that there was no constant relation between the lesions in tonsils, cervical lymph nodes and lungs. Wu Lien-teh also drew attention to the fact that cervical buboes sometimes predominated in mild outbreaks of plague. It is also noteworthy that axillary buboes were frequent in Transbaikalia, especially in patients infected through tarabagan fleas or through handling infected rodents, and yet the incidence of secondary lung involvement was not particularly high.

Far more important still than such considerations is the fact that the lung lesions usually found in bubonic plague, and carefully studied by numerous investigators, could as a rule be definitely ascribed to an invasion of the lungs through the blood stream.

Statements as to the frequency of secondary lung involvement by this usual route vary much and it is difficult to collate them because they are based upon different methods of clinical or postmortem examination. It has been maintained with much reason that some involvement of the respiratory tract is usually if not invariably present in bubonic infections followed by bacteraemia, varying in degree from simple catarrh to grave forms of congestion and specific bronchopneumonia. Di Mattei,[5] a strenuous advocate of this idea, could demonstrate *P. pestis* in the sputum of patients in whom no definite signs of pulmonary localization could be detected and even recommended sputum examination as a suitable means for the early bacteriological diagnosis of bubonic plague infection.

Circulatory system. Frequently subserous haemorrhages are found on the inner layer of the pericardium and the epicardium. Some accumulation of liquid in the pericardial sac (usually clear and without admixture of blood) is quite common.

The heart, especially on the right side, is usually distended and filled with dark red blood showing little tendency to coagulate. Haemorrhages under the endocardium and the intima of the valves are occasionally noted. The heart muscle constantly presents alterations corresponding to cloudy swelling or fatty degeneration.

The conspicuous changes taking place in the veins located in the vicinity of buboes have already been noted (page 206). Small haemorrhages are frequently found under the intima of the veins in general. Vint [115] stressed that the haemorrhages found in plague victims were jet black in colour, thus contrasting with the bright red petechiae usually met with in septic conditions due to other causes.

Spleen. The spleen, though usually enlarged, is in a considerable minority of cases not much more than normal in size. It is further noteworthy that in plague the organ is often distinctly firmer in consistency than in other infectious diseases. It would seem that both enlargement and softening are more marked in instances of rapidly progressing infection; this holds true of cases with inconspicuous buboes as well as of instances of respiratory infection where no manifest pneumonia develops.

The capsule of the organ is tense and more or less opaque; subcapsular haemorrhages are frequent. The substance of the spleen shows some congestion. The follicles, if at all visible, are small, not rarely surrounded by a ring of intensely congested tissue; the trabeculae usually not prominent. Anaemic infarcts are not infrequent.

Liver. The liver is often enlarged and, as a rule, more or less congested. Small haemorrhages may be present below the capsule. Usually there is evidence of cloudy swelling or early fatty degeneration. Sometimes a nutmeg appearance has been noted, but it seems an open question whether this is due to venous congestion or to an acute degeneration of the liver cells round the hepatic veins (Wu Lien-teh & Woodhead [128]).

Besides these generalized alterations, many observers described nodes or spots of a yellowish colour either on the surface or in the parenchyma of the liver, due apparently to a localized fatty degeneration or to necrosis.

Kidneys. The kidneys show more or less advanced degenerative changes, sometimes true nephritis. Haemorrhages are frequently noted on the inner layer of the capsule and in the pelvis, more rarely on the surface of the organ. Smaller necrotic foci have been observed but are infrequent.

Gastro-intestinal tract. In the overwhelming majority of autopsies no marked changes are found in the gastro-intestinal apparatus. Haemorrhages beneath the peritoneal coats are comparatively rare, but are frequently noted beneath the mucosa of the stomach and intestines. Not rarely hyperaemia of part of the mucosa of the stomach or intestines, or even congestion of some portion of the intestines throughout all layers, is

present A catarrhal condition sometimes accompanied in the intestines by a pinkish excretion is not infrequent

Advanced changes especially ulcerations were described by a few observers only In some of these cases at least it is doubtful whether such unusual lesions were actually specific in nature

Brain and meninges In addition to the congestion and oedema of the brain and meninges usually met with at autopsy instances of plague meningitis have been described by several workers Although the meningeal process was usually due to a secondary invasion through the blood stream subsequent to bubonic infection a few authors (Lafont et al [61] Williams,[129] Lewillon et al, [65] and Landsborough & Tunnell [6]) claimed to have observed a primary form of plague meningitis One cannot help feeling however, that in these instances ill defined or hidden buboes might have escaped detection

Landsborough & Tunnell [6] described the cerebrospinal lesions found at autopsy of their case as follows

Brain congested convolutions and sulci flattened tissue soft thick canary yellow fibrino purulent exudate covering most of the external surface of the brain ventricles especially the lateral ones dilated and containing plenty of turbid fluid spinal cord showing marked congestion but less exudate except in the region of the cauda equina

As mentioned before, a very unusual case of chronic relapsing latent plague meningitis was described by Meyer et al [78]

Pathology of Primary Pneumonic Plague

Since as has been mentioned before (page 207) plague infection entering through the deep parts of the respiratory system does not invariably lead to the formation of pneumonic foci but may result in a rapidly generalized infection with only insignificant changes (particularly congestion and oedema of the lungs) in the respiratory tract it would be preferable to speak of pulmonary rather than 'primary pneumonic plague—the more so because not infrequently it is doubtful whether authors dealing with the problems of pneumonic plague refer to the primary type or to secondary lung invasions taking place through the blood stream On the other hand, the term primary pneumonic plague has become so familiar that one must be hesitant to replace it desirable though this would be

Pathogenesis

While most investigators believe that primary pneumonic (pulmonary) plague is caused by an infection entering through the deeper portion of the respiratory tract, a few, especially Koulecha [60] considered the tonsils or other parts of the upper respiratory tract as the portal of entry of the infection which then reached the lungs through the blood stream As

discussed earlier (page 207) experimental observations did not support this "tonsillar" theory and the same holds true of the findings in man.

(1) In instances where an infection initially entering the tonsils led to pneumonia, cervical buboes, if not pre-existent, invariably developed in the course of the disease. In primary pneumonic plague the cervical lymph-nodes showed slight or even no changes. In the experience of most investigators the lesions in the upper respiratory tract as well were as a rule slight and obviously secondary in nature.

(2) As shown by the work of Girard,[31] who made parallel examinations of the sputum and blood cultures in a series of pneumonic cases, plague bacilli were present in the former before they could be demonstrated in the blood.

Identical findings were recently recorded by Wagle & Bedarkar,[116] while Wu Lien-teh[1, 4] and, more recently, Hennessey[48] referred to instances of primary pneumonic plague where even post mortem no bacteriaemia was demonstrable.

(3) Histological examinations also convinced most investigators that the lung lesions in primary pneumonic plague evolved initially and were followed, instead of being preceded, by an invasion of the blood stream. Specially noteworthy is a record of Jettmar[5] concerning a patient who committed suicide early in the disease. Here the blood stream was still free from plague bacilli and it could be seen that these micro-organisms were just on the point of penetrating into the blood vessels through the lymph spaces.

Nattan-Larrier & Richard[8] came to the conclusion that in man as in experimentally infected animals a bacterial alveolitis was the characteristic feature of primary pneumonic plague, the infection propagating from the respiratory lumina towards the lymphatics.

Pathology

Pneumonic plague is distinguished from the bubonic type by a more rapid course and—except in patients treated with the now available potent remedies—by an almost invariably fatal termination.

In order to explain these differences it must be kept in mind that in bubonic plague the defensive mechanisms of the body are apt to prevent or at least to retard or modify a massive generalized infection. Considering the problem of secondary pneumonic plague in particular, Heckenroth[47] maintained that this type of lung involvement was caused by plague bacilli attenuated in virulence by the antibodies produced in the primary bubo.

In primary pneumonic plague, on the other hand, the initially invaded area is devoid or nearly devoid of defensive means (Wu Lien-teh[1, 4]), so that the morbid process can spread without hindrance, leading to an enormous agglomeration of the bacilli in the lungs. Even apart from the fact that bacteraemia, though undoubtedly secondary in nature, develops fairly

soon, a most ominous role is played by an early and massive absorption of endotoxins van Loon[67] aptly comparing the condition in primary pneumonic plague to that in acute peritonitis Wu Lien teh[1 1] was therefore right in maintaining that the surprising thing was not that so many patients succumbed to the former disease but that even a few should recover

Morbid anatomy

In dealing with the morbid anatomy of primary pneumonic plague, it is sufficient to consider the lesions in the respiratory tract only, because appearances in the other organs are identical with those seen in victims to bubonic infection

Upper respiratory passages The changes found in the upper respiratory passages of victims to primary pneumonic plague are usually not prominent thus being similar to those generally noted in the bubonic type

Trachea and bronchi In well marked cases the mucosa of the trachea and bronchi is markedly congested The mucous membrane of the trachea is covered by a blood stained, sometimes frothy exudate The bronchi occasionally show a similar covering, more often they contain a bloody and frothy serous fluid Submucous haemorrhages are occasionally seen

More advanced lesions are rare but small necrotic patches were some times noted On the other hand, in less characteristic cases the trachea may be pale and may contain non bloody mucus or mucopurulent secretion sometimes of a brownish colour In cases without manifest pneumonia, secretion may be totally absent but some degree of congestion is invariably noted

Pleurae Recent fibrinous adhesions between both layers of the pleurae are frequent, but are generally present over the inflamed parts of the lungs only The presence of any quantity of serous exudate whether blood stained or not is comparatively rare

Fibrinous deposits are often present over the surface of pneumonic areas though varying much in extent and appearance Purulent exudate on the other hand, seems to be practically absent except in cases complicating pre existing tuberculosis

Haemorrhages of different sizes, rarely present beneath the parietal layer of the pleura, are very often noted beneath the visceral pleura Sometimes they are scattered all over this or localized at areas free from pneumonic changes In well marked cases they are often concentrated above superficial pneumonic patches and form together with the fibrin deposits a rather characteristic pattern of red and yellow dots and bands first described by Albrecht & Ghon[1] - However, insignificant changes may be present even over superficially situated pneumonic foci while in the case of their more central localization macroscopic alterations of the pleurae may be altogether absent

Lungs. As postulated by workers such as Vint[115] and Wright[112] a distinction may be made between three types of lung manifestations met with in primary pneumonic or, one should rather say, pulmonary plague:

(*a*) A form in which acute congestion and usually also lung oedema are marked but where, though plague bacilli abound, no consolidation is present.

(*b*) A type characterized by the presence of lobular pneumonic foci.

(*c*) A form where lobar involvement seems to be present.

Wright also referred to a fourth type characterized by the presence of scattered and apparently necrotic nodules, but this process, also referred to by Vint[11] and by Hennessey[4], was obviously secondary in nature, being due to an embolic invasion of the lungs through the blood stream.

Hennessey[4] maintained that whereas formerly instances of "broncho-lobular" pneumonic plague had predominated in Uganda, since 1933 the apparently lobar form was preponderant. He believed that the prevalence of this type was due to a loss of virulence of the local plague strains which had become manifest at the same time. It must be emphasized, however, that most workers who had access to sufficiently large material noted the simultaneous presence of cases with lobular and cases with so-called "lobar" pneumonic changes during the pneumonic plague epidemics they observed. More than that, though it is convenient to adopt for descriptive purposes the classification given above, actually no sharp line of demarcation can be drawn between the three types of morbid appearances, a transitory group with slight pneumonic lesions standing between the first and second, while, as discussed below, the so-called lobar form represents merely an advanced stage due to the confluence of originally lobular pneumonic foci.

The lobular foci encountered in primary pneumonic plague may be situated either near the surface or within the lobes of the lungs. One or several such patches may be present in one lobe; sometimes they were found to be arranged along one bronchus "like the flowers of the hydrangea" (Strong[110]).

When situated near the surface, the lobular foci bulge somewhat and the adjacent pleura shows frequently the peculiar changes described above. On cut section the foci appear wedge-shaped or round and are often surrounded by a zone of engorgement and oedema. Their colour is usually either dark or greyish red in the centre with a deep red periphery; only in rare instances do they show a uniformly grey colour. As in pneumonic plague in general, the cut section does not show the definite granulation typical of croupous pneumonia; no fibrin can be scraped off, but only a uniform greyish white fluid. The foci situated away from the surface of the lungs are, as a rule, roundish in shape. They display in well marked cases the same characteristics as the superficial foci, but in instances with slight pneumonia they may be ill defined.

In the opinion of most observers, the so called lobar consolidations found in primary pneumonic plague are due to a confluence of originally lobular foci It is therefore not surprising that grey hepatization of a whole lobe, so frequently seen in croupous pneumonia is never met with in pneumonic plague and that even extended areas of red hepatization are rarely present As a rule the extensively affected lobes show on cut section a variegated aspect areas of grey hepatization adjoining those with red hepatization and these in turn shading into parts of the lung tissue which are merely in a stage of engorgement (Strong [110]) Both lobular foci and more extensive pneumonic areas may be present side by side in the same lung or even lobe

Bronchial lymph nodes Although in some cases the bronchial lymph nodes may exhibit little or no change, they are usually markedly enlarged, soft, and hyperaemic, on cut section they may show punctiform ecchymoses or a general deepening of colour as a result of diffuse haemorrhage Koulecha [60] was of the opinion that the affected lymph nodes finally displayed the appearances of Albrecht & Ghon s primary buboes of the secondary order

The tissues surrounding the lymph nodes at the bifurcation of the trachea are often oedematous and show haemorrhages, but advanced periadenitis is absent The oedema with extensive haemorrhages frequently noted by Strong [110] during the 1910 1 Manchurian epidemic, in the anterior mediastinum round the thymus gland was not conspicuous in the victims of primary pneumonic plague dissected by Pollitzer during the 1920-1 outbreak

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Chapter 5

METHODS OF LABORATORY DIAGNOSIS

EXAMINATION OF RODENTS AND FLEAS

Before discussing the laboratory methods available for the detection of plague in individual rodents, it should be pointed out that a properly functioning intelligence service is of outstanding value for this work, since the laboratory staff is thus kept informed of the occurrence and extent of unusual rodent mortality. In the case of the commensal rodents in particular, the finding of an appreciable number of animals which have succumbed spontaneously may ordinarily be taken as prima facie evidence for the existence of a plague epizootic, while marked scarcity of live rodents in a locality which previously showed heavy infestation is indicative of a past major epizootic. In view of what has been discussed in chapter 4, every possible effort must be made to examine the rodents found dead, because they offer the strongest chance of obtaining frankly positive evidence of infection. This point is well illustrated by the recently published observations made during the period 1940-50 by Gross & Bonnet [49] in the Hamakua district of Hawaii. These workers detected plague 358 times in 4,805 rodents found dead, as against 23 times in 20,623 killed animals and 20 times in 304,807 trapped rodents.

Evaluation of Methods

The principal methods used to detect plague infection in rodents are as follows:

(1) autopsy;

(2) bacteriological tests (smears and cultures);

(3) serological tests;

(4) animal experiments with material from individual rodents or with cultures isolated from them.

(5) animal experiments with pooled organs,
(6) animal experiments with pooled fleas

Autopsy

Bearing in mind that the pioneer work of the Plague Research Commission [88] was done at the time when acute plague was rampant among the rats of India, it is not surprising to find that the Commissioners comparing in 1907 the various methods available for the diagnosis of rodent plague, emphasized the outstanding value of the macroscopic findings In the opinion of the Plague Commission, it was justifiable to diagnose the disease in rats on the basis of the presence of even one of the pathognomonic gross signs, regardless of whether or not subsequent smear examinations culture tests, or animal experiments yielded positive results

It is no longer possible to evaluate the results of macroscopic examinations as highly as the Plague Research Commission did—not only because animals may be found which, for the reasons set forth in the preceding chapter, show no gross signs of the infection, but also because observations made outside India have proved that morbid appearances macroscopically indistinguishable from plague may be produced by infective agents other than *Pasteurella pestis* It is noteworthy in this connexion that, in the experience of Macchiavello [70] at Antofagasta, Chile rats which afterwards proved to be plague infected were less apt to show suggestive gross signs than a large number of macroscopically suspect but actually plague negative animals

Nevertheless the presence of signs such as typical buboes a characteristic mottled or waxlike appearance of the liver, and clear pleural fluid has been found to be strongly suggestive in most plague areas as has been noted before frequency of rats showing such pathognomonic signs proves the presence of an acute epizootic while their rarity is suggestive of a quiescent stage of the infection In order to make reliable macroscopic observations, it is necessary, however, to perform dissection before putrefaction has set in

Bacteriological tests

While subsequent workers following the lead of the Plague Research Commission continued to lay stress upon the direct results of rat dissection they usually considered it necessary to confirm the diagnosis with the aid of laboratory methods, especially smear examination and culture tests

Although, as will be discussed later (page 237), great care has to be exerted in interpreting the results of smear examination the abundance of characteristic bipolar stained bacilli or involution forms in smears prepared from the organs of the dissected rodents goes a long way towards supporting a positive diagnosis in the course of outbreaks previously confirmed by fully reliable methods

Culture methods are fully satisfactory whenever it is possible to examine fresh material but the presence of putrefaction renders it difficult or even

impossible to obtain reliable results from the buboes or internal organs of plague-suspect animals by such tests. However, the possibility of proving the existence of plague in putrefied rodents was greatly increased by the recommendation, first made in 1926 by Pons (quoted by Murdock [illegible]), then by Fusco (quoted by Devignat [illegible]) and Micheletti [illegible] and again independently by Uriarte et al.[111], that in such cases the bone marrow of the tibia or femur rather than the internal organs should be used for bacteriological examinations.[a]

While the great value of this method was confirmed by most field workers and also through experiments made with rat and guinea-pig carcasses by Russo[81], a few investigators came to less satisfactory conclusions. Macchiavello & Paracampo[3] in particular, working in the hot climate of north-east Brazil, had many failures with this procedure and expressed an urgent warning against the exclusion of the possibility of plague on the grounds of negative results thus obtained. They admitted, however, that the impossibility of cultivating *P. pestis* from many of their rodents appeared to be largely due to the fact that the plague strains prevailing in north-east Brazil were often too attenuated to cause septicaemia. Bone-marrow cultures made from animals suffering from acute or subacute plague were invariably positive and less contaminated than those from internal organs. Moreover, since the viscera of dead rodents were apt to be destroyed by insect larvae, bone-marrow examination was often the only method practicable for laboratory investigation.

Serological tests

Since serological tests yield results far more rapidly than do culture tests, they are of outstanding value for the confirmation of the prima facie evidence obtained from dissection and smear examination. The methods suitable for such tests have been referred to in Chapter 3 and are dealt with in greater detail on page 243.

Animal experiments

Individual tests. Animal experiments performed with the organs of individual rodents or with the growths isolated from them yield fully reliable results if conducted in an adequate manner. They are therefore of the greatest value in confirming the diagnosis both in incipient plague epizootics and in sporadic instances of rodent infection. However, it is hardly possible to perform experiments with material from each individual animal during the routine examination of large numbers of rodents.

[a] Estragnin, while endorsing the value of bone-marrow examination, considered it still more advantageous to culture the brain of putrefied rats in a medium consisting of eight parts of ordinary and two parts of liver bouillon.

Pooling tests

(*a*) *Organs* A most important innovation permitting of the large scale use of animal experimentation for the diagnosis of rodent plague, was introduced by Chapin [19] in 1909 Finding that many of the rats dissected by him at Seattle USA, were too putrified to show gross lesions, and wishing not to overlook instances of inapparent infection he resorted to cutaneous inoculation of guinea pigs with the organs of not one but a whole group of rats Chapin himself perhaps because he used the organs of every rat of an entire catch sometimes 30 or even more in number to infect single guinea pigs, failed to obtain positive results in the 400 rats tested in this manner However, the method soon afterwards proved successful in the hands of other workers, such as Lloyd [11, 68] (also at Seattle) in 1914 and Creel [11] at New Orleans in 1915, and has been much used since

Swellengrebel & Hoesen [104] seem to have been the first to replace Chapin s method by subcutaneous infection of guinea pigs with suspensions obtained by collecting small pieces of the organs of the rats to be examined in a sterile mortar and grinding up this material with a little sterile saline Handling in this manner 7,625 apparently normal rats, the two workers were able to detect plague in 54 pools

The value of this method which has remained one of the standard procedures for ascertaining the presence of plague in commensal rats as well as in wild rodents, has been well demonstrated by the following results reported at the 1934 Calcutta Conference of Medical Research Workers (quoted by Pollitzer [89])

Species	*Number of animals used o group tests*	*Number of animals found smear positive*	*Number of groups tested*	*Number of positi e group tests*
Rattus rattus	2 107	2	141	13
Mus musculus	1 393	—	100	6
Bandicota malabarica	306	1	95	15

These results are all the more noteworthy because rodents showing suggestive signs of infection were not included in the group tests Cultures made from the suspensions prepared for guinea pig infection were often unsatisfactory

An interesting method, taking advantage of bone marrow examination as well as of pooling was used by Devignat [5] in the course of monthly rodent surveys in the Belgian Congo The procedure consisted in cutting off the legs of freshly killed rodents (mainly *Rattus natalensis*) just above the knee, aspirating the bone marrow with the aid of a syringe provided with a suitably thin and short bevelled needle, and suspending the material in a bottle containing 3 ml of sterile normal saline Repeating this operation with each animal of the daily catch it was thus possible to

obtain a pool containing material from up to 500 rodents. The suspension was immediately used for subcutaneous inoculation of a guinea pig, which was then dispatched to the laboratory. In the case of animals the bones of which were too small to yield bone marrow, the spleens were successively used for cutaneous inoculation of a guinea pig.

Practising these methods, Devignat repeatedly succeeded in demonstrating the existence of plague in rodents which otherwise appeared to be free from the infection.

Van Riel & Mol[*], working in the Kivu region of the Belgian Congo, also obtained good results with bone marrow pools. They used the subcutaneous method for infecting guinea pigs in the field, but preferred the cutaneous route when making tests in the laboratory.

It deserves attention, however, that the experiences made by Heisch[53] in the Rongai area of Kenya, where inapparent plague seemed to be prevalent among the wild rodents, did not confirm the value of the above-described method. Heisch referred in particular to one instance where the pooled spleens, bone marrows, and fleas collected from a batch of *Arvicanthis* had been used separately for the inoculation of different guinea pigs, and positive results had been obtained only with the spleens. He urged, therefore, that pools from the internal organs rather than those from the bone marrow should be used to demonstrate the presence of inapparent rodent plague and recommended in particular the technique adopted by Meyer et al.[7], who inoculated their test guinea pigs with material collected from not more than five plague-suspect ground squirrels.

(*b*) *Fleas*. Several observers like Kitasato, the Plague Research Commission, Petrie & Todd and Jettmar (quoted by Pollitzer[82]) had shown that while, particularly at the end of an intense epizootic, it was often difficult or even impossible to find plague-affected rodents, it was usually comparatively easy to collect infected fleas for laboratory tests. However, since smear examination and culture methods are, as a rule, rather unsuitable for demonstrating the existence of the infection in these insects, it was often necessary to resort to animal experiments in order to get reliable results.

An early attempt to use pooling methods when examining fleas was made by Swellengrebel & Hoesen[101] who, working with 15 279 fleas, obtained positive results with 18 pools. However, the method attracted no attention until it was once more applied in 1936 by Eskey[3] to detect foci of wild rodent plague in California. Since then ample use has been made of this procedure in order to ascertain the presence of the infection in commensal rats as well as in wild rodents.

No doubt can exist that the large-scale use of one or preferably both of the above-mentioned pooling methods is alone fully reliable for deciding whether or not plague is present in a given rodent population.

Pooling tests

(*a*) *Organs* A most important innovation, permitting of the large scale use of animal experimentation for the diagnosis of rodent plague was introduced by Chapin [19] in 1909 Finding that many of the rats dissected by him at Seattle, USA, were too putrified to show gross lesions, and wishing not to overlook instances of inapparent infection, he resorted to cutaneous inoculation of guinea pigs with the organs of not one but a whole group of rats Chapin himself, perhaps because he used the organs of every rat of an entire catch sometimes 30 or even more in number" to infect single guinea pigs, failed to obtain positive results in the 400 rats tested in this manner However, the method soon afterwards proved successful in the hands of other workers, such as Lloyd [21, 68] (also at Seattle) in 1914 and Creel [11] at New Orleans in 1915 and has been much used since

Swellengrebel & Hoesen [101] seem to have been the first to replace Chapin s method by subcutaneous infection of guinea pigs with suspensions obtained by collecting small pieces of the organs of the rats to be examined in a sterile mortar and grinding up this material with a little sterile saline Handling in this manner 7,625 apparently normal rats the two workers were able to detect plague in 54 pools

The value of this method, which has remained one of the standard procedures for ascertaining the presence of plague in commensal rats as well as in wild rodents, has been well demonstrated by the following results reported at the 1934 Calcutta Conference of Medical Research Workers (quoted by Pollitzer [89])

Species	*Number of animals used or group tests*	*Number of animals found smear positive*	*Number of groups tested*	*Number of positive group tests*
Rattus rattus	2 107	2	141	13
Mus musculus	1 393	—	100	6
Bandicota malabarica	306	1	95	15

These results are all the more noteworthy because rodents showing suggestive signs of infection were not included in the group tests Cultures *made from the suspensions prepared for guinea pig infection* were often unsatisfactory

An interesting method, taking advantage of bone marrow examination as well as of pooling was used by Devignat in the course of monthly rodent surveys in the Belgian Congo The procedure consisted in cutting off the legs of freshly killed rodents (mainly *Rattus natalensis*) just above the knee, aspirating the bone marrow with the aid of a syringe provided with a suitably thin and short bevelled needle, and suspending the material in a bottle containing 3 ml of sterile normal saline Repeating this operation with each animal of the daily catch it was thus possible to

obtain a pool containing material from up to 500 rodents. The suspension was immediately used for subcutaneous inoculation of a guinea pig which was then dispatched to the laboratory. In the case of animals the bones of which were too small to yield bone marrow, the spleens were successively used for cutaneous inoculation of a guinea pig.

Practising these methods, Devignat repeatedly succeeded in demonstrating the existence of plague in rodents which otherwise appeared to be free from the infection.

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It deserves attention however that the experiences made by Heisch[3] in the Rongai area of Kenya where inapparent plague seemed to be prevalent among the wild rodents did not confirm the value of the above described method. Heisch referred in particular to one instance where the pooled spleens, bone marrows and fleas collected from a batch of *Arvicanthus* had been used separately for the inoculation of different guinea pigs, and positive results had been obtained only with the spleens. He urged therefore that pools from the internal organs rather than those from the bone marrow should be used to demonstrate the presence of inapparent rodent plague and recommended in particular the technique adopted by Meyer et al[7] who inoculated their test guinea pigs with material collected from not more than five plague suspect ground squirrels.

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No doubt can exist that the large scale use of one or preferably both of the above mentioned pooling methods is alone fully reliable for deciding whether or not plague is present in a given rodent population.

Comparison Assessing the value of pooling tests in comparison with other methods of rodent and flea examination, it must be realized that, paradoxical though it may seem at first glance, the extreme sensitivity of the pooling tests limits their usefulness Being apt to prove positive in the case of pools containing merely a few, or possibly even single virulent plague bacilli, they furnish a reliable answer to the question of whether plague is present in the rodents or fleas examined, but do not show to what extent the infection is present Although this second point may be rather immaterial under certain circumstances—for instance when making large scale wild rodent surveys—it is of paramount importance when dealing with plague manifestations in individual localities where the proper conduct of anti epidemic work depends on full awareness of the trend of infection Under these circumstances, to neglect the methods available for the examination of individual rodents in favour of pooling tests would be erroneous

As has been noted above, the method of using flea pools has the great advantage of being applicable even when the number of rodents has become much reduced by an extensive epizootic Moreover, since fleas are not merely vectors but also to a considerable extent reservoirs of plague, positive results with flea pools may be obtained during the off seasons when it may be difficult or even impossible to prove the continued existence of rodent infection It must also be admitted that examination of flea pools is more expedient and less dangerous than that of tissue pools

It is therefore not surprising to find that some workers place sole reliance on the former method, considering the routine examination of tissue pools to be generally superfluous Humphreys et al [57] recently supported this opinion because they found that parallel examination of 5 019 tissue pools and 4 641 flea pools yielded only three instances in which the former proved positive and the latter negative However, pointing out that in two of these three instances only one flea per animal had been obtained, Humphreys and his co workers admitted the value of tissue pool tests in cases where the rodents concerned were free, or nearly free from ectoparasites It must be realized as well that positive results with flea pools may prove misleading in so far as the rodents which appear to be concerned may have picked up fleas from plague-susceptible species without suffering from the disease themselves It would appear, therefore, that examination of tissue pools since it serves to check the results obtained with flea pools should be used parallel with flea pool tests periodically at least, if not as a matter of routine

Techniques

Dealing with the technique of rodent examination, it seems permissible to concentrate attention on the methods suitable for commensal rats,

which in most plague areas form the bulk of the material to be handled the more so because the procedures to be adopted in the case of other rodent species do not differ in principle from those required for rat investigation

Collection and delivery of rats

If rats found dead or killed by the people are available for examination they should preferably be put into individual paper bags or be individually wrapped in paper for transport to the laboratory

An alternative adopted in China was to place large tin buckets or wooden boxes with lids or large covered earthenware jars at strategic points of the plague affected settlements The people were urged to place the dead rodents in these collecting vessels which were inspected twice daily the rodents found were taken to the laboratory in covered tin boxes In plague affected localities where the people were un-co operative it was possible to obtain dead rats by paying rewards to the scavengers for the delivery of carcasses found by them in refuse

Rats caught in snap traps should be transported to the laboratory in individual paper or canvas bags An adequate procedure recently recommended in this connexion by Gross & Bonnet [49] was as follows taking care not to touch the animals the trappers freed them by picking up the trap by the base and releasing the striker allowing the rodent to fall into a suitable receptacle If it was proposed to examine the fleas the retrieved rodent was placed in a paper bag and one-quarter of a teaspoonful of calcium cyanide was inserted the bag was then shaken and tightly tied by the neck Otherwise the trapped rodents were put into gallon-cans containing kerosene

Cage traps in which rats have been caught alive should be enclosed in canvas bags for transport to the laboratory

Killing rats trapped alive

Although as discussed by Omar [a] a considerable number of procedures has been recommended for killing rats delivered live to the laboratory the following deserve prime consideration

Submersion While the method of submerging the rodents in water or other fluids is the least attractive of the available procedures it is cheap and effective the more so as it facilitates collection of the fleas harboured by the rodents The use of antiseptic fluids in place of pure water offers no essential advantages and must be avoided when it is planned to use the fleas for culture examination or pooling tests

Volatile fluids A simple and effective method for killing rats in cage traps enclosed in canvas bags is to put each bag with the trap in [illegible] a suitably sized box or tin partly to open the bag and to push in a [illegible]

soaked in a volatile fluid such as chloroform, ether, or gasolene The box or tin is then kept closed for about 20 minutes, a time sufficient to kill the rat and at least to stun its fleas Gasolene is the cheapest of the suitable volatile fluids and is also preferable in so far as, according to Eskey & Haas,[31] chloroform or ether is apt to exert an adverse influence on the infectivity of the fleas

Lethal gases Trapped rats as well as their fleas may be conveniently killed with the aid of calcium cyanide For this purpose the cage traps in their canvas bags, which should first be opened up are put into a tin or wooden box provided with a well fitting cover and an opening suitable for pumping in the dust after the receptacle has been closed

The rats and their fleas are killed rapidly and, if a tin of sufficient size is provided, several traps may be dealt with at the same time

Calcium cyanide is also suitable for dealing with dead rats delivered to the laboratory, particularly those not enclosed in bags For this purpose a layer of the chemical is put into a large tin bucket provided with a well fitting cover The rat carcasses are placed on the calcium cyanide and should preferably be kept in the closed tin overnight Before they are taken out in the morning the tin should be left open for at least 15 minutes In the case of an acute epizootic, calcium cyanide may be applied once more before ventilation of the tin

To avoid accidents, these methods should be applied in the open air by workers trained in their use

Collection of fleas

Before carrying out dissections, it is necessary to free the rodents from fleas and other ectoparasites This can be easily done by thoroughly brushing and combing all parts of the fur of the carcasses These operations are best performed either in a large, deep, white enamel pan or while holding or suspending the rats over a large, white enamel basin filled with water Before starting the work, the rats may be first plunged into the water, some workers prefer to keep them submerged while they are brushed and combed

The fleas and other ectoparasites collected from each rat should be put into a separate stoppered ampoule to await classification and indexing

Dissection and preparation of material

It would be redundant to deal in detail with the technique of rat dissection which is performed according to the methods generally adopted for the autopsy of small laboratory animals—although as is described on page 245 with special precautions However, attention must be drawn to the necessity of examining with special care the lymph nodes of plague suspect carcasses The recommendation of the Plague Research Commission that

the pectoral muscles should be cut so as to make the axillary lymph nodes visible has been referred to in Chapter 4 and the advice of the Commission to inspect the cut surface even of lymph nodes which appear to be unaffected externally has also been noted In addition to the superficial lymph nodes the deep cervical mesenterial retroperitoneal and pelvic lymph nodes should receive attention

Smears or impression films should be made not only from abnormal lymph nodes but also from the heart blood spleen liver and lungs all of which should be placed on one slide in order to save labour and material Cultures and material for animal experiments or pooling tests should be taken from any bubo present as well as from one or more of the above mentioned internal organs or in the case of putrefaction from the bone marrow

Recording of results

As recommended in the antiplague instructions published by the Office International d Hygiène Publique in 1937[82] daily records should be kept showing (*a*) the number of rats and other rodents examined classified by species (*b*) the number of males females and pregnant females in each species (*c*) the number of rats found infected in each species

These figures and their totals are used to calculate plague indices i e the percentages of rodents found infected in each species and in the totals If as is advisable graphs based on these values are prepared it is preferable to use for this purpose the five day averages instead of the daily figures (Shih & Pollitzer[96])

Flea examination

While animal experiments with pooled fleas are of outstanding value in plague work possibilities for taking advantage of bacteriological methods for the examination of plague suspect fleas are rather limited It has been stated by Webster[113] that smears of individual crushed fleas may be stained and examined for the presence of plague like bacilli but only a proportion of the plague infected fleas will be detected thus and the morphology is not really conclusive Studying the suitability of culture methods for detecting plague in fleas Tiflov[106] worked with suspensions prepared by crushing 26 100 living wild rodent fleas (*Nosopsyllus consimilis* or *Ctenophthalmus wagneri*) each batch containing but one infected flea Results obtained on the one hand by injecting part of the suspensions intramuscularly into guinea pigs and on the other by cultivation were as follows

Method	Number [illegible]	[illegible]
Guinea pig infection	43	[illegible]
Cultivation on agar	39	33
Cultivation on blood agar	39	[illegible]

However, results obtained by most other workers when using suspensions prepared by crushing fleas for cultivation were so disappointing that it was recommended that the proventriculus and stomach of plague suspect fleas should be dissected out and the contents used for cultivation. Satisfactory results were obtained in this way both with individual fleas (Gore[4]) and when pooling the stomach contents of 60-70 fleas (Bichkov & Borzenkov[10]), but this procedure is far too elaborate to be suitable for routine work. Animal experiments therefore remain the method of choice for routine examination of plague suspect fleas.

EXAMINATION IN HUMAN PLAGUE

Patients

Bubonic plague

Puncture. Although ample use has been made of bubo puncture to collect material for the bacteriological diagnosis of bubonic plague, various objections have been raised against this method.

Some workers pointed out that in the acute stage of the disease it was difficult, with the aid of this procedure, to obtain sufficient material for examination. It must be noted, however, that this difficulty may be easily overcome if, as recommended by Girard,[39] the needle and syringe are well washed out after puncture with a little saline and the latter is then used for the tests. The alternative sometimes suggested of first injecting a few drops of sterile saline or broth into the substance of the bubo and then aspirating, is less recommendable—if for no other reason than that this procedure considerably adds to the discomfort of the patient.

Another objection was that even in the acute stage of the disease the method was apt to yield negative results if, instead of the affected lymph nodes themselves, the often conspicuous oedema hiding them were punctured.[b] It was also pointed out that since in the course of recovery, the causative organisms in the affected lymph nodes first became scanty and then altogether disappeared, punctures carried out late in the disease often failed to confirm the diagnosis of plague. This held true particularly of suppurated buboes.

The fear was moreover expressed that puncture of plague buboes might prove harmful by facilitating entry of the infection into the lymph- or bloodstream. It is difficult to agree with this opinion if it is considered that extirpation of plague buboes has been successfully practised to treat the disease; it must, however, be admitted that the operation is rather painful.

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[b] As aptly pointed out by de Smidt, the punctate of plague buboes should contain numerous cells as well as plague bacilli. Absence of the former indicates that the buboes have not been reached.

Blood culture Dissatisfied with the method of bubo puncture several observers advised the use, instead of early blood culture for the diagnosis of bubonic plague Some such as Bonebakker,[14] recommended bile media for this purpose South American workers such as da Silva [95] and Barreto & Castro [8] preferred direct intraperitoneal inoculation of experimental animals with the blood of the patients

As has been pointed out in chapter 4 particularly rapid results may be obtained with the latter procedure if according to the proposal of Gotschlich,[48] material for microscopic and culture examinations is taken in vivo from the peritoneal cavity of the infected animals

In addition to the above mentioned methods serodiagnostic procedures may be used (see page 243)

Evaluation When trying to determine the comparative value of these methods it must be kept in mind that, in order to take full advantage of the potent remedies now available for plague treatment by starting their administration at the first possible moment an early diagnosis of the disease is indispensable Considering that in bubonic plague development of the gland affection precedes that of septicaemia it would seem at first glance that examination of material obtained through bubo puncture should be of greater value for making a quick diagnosis than blood tests and still more than serodiagnostic procedures which yield a positive result only after the morbid process has developed It must be realized however that this holds true only as far as smear examination of the bubo contents is concerned because confirmation of the diagnosis through culture tests and animal experiments is no less time-consuming when such material is used than when the blood of the patient is examined

The finding of numerous and characteristic bacilli in smears made from the buboes goes a long way towards supporting the diagnosis but it is questionable how far such results are superior in value to the prima facie evidence procurable in typical cases through clinical examination alone It is felt therefore that bubo puncture followed by smear examination and confirmatory tests should be used only in clinically doubtful cases or in order to establish the diagnosis in hitherto plague free localities Ordinarily a decision whether or not to initiate treatment can be reached on clinical grounds and blood examination or (particularly in mild cases or those which have received early treatment) serodiagnostic methods should be used to confirm the diagnosis

Primary pneumonic plague

A clinical diagnosis of primary pneumonic plague is easy as soon as the characteristic sputum is expectorated and this evidence is [illegible] supported by the examination of sputum smears which in typical cases show an abundance of characteristic bacilli Culture tests and animal

experiments carried out with such sputum yield comparatively rapid results

As will be discussed later, primary pneumonic plague is ushered in by a stage lasting about 20 24 hours, during which cough is absent or insignificant and no characteristic expectoration occurs At this stage, plague bacilli are either altogether absent from the sputum or saliva, or are present in such small numbers among other bacteria as not to be recognizable with certainty Clinically, such patients show signs of an acute general infection but no lung involvement To arrive at a definite diagnosis at this stage of the disease is therefore difficult or impossible If plague is present, further clinical observation as well as repetition of sputum examinations at frequent intervals will eventually lead to a diagnosis It is clear, however that during epidemics, and particularly when dealing with patients who have been in contact with pneumonic plague victims, it is imperative not to wait for such confirmation but to start energetic treatment as soon as the presence of the disease is suspected

Septicaemic plague

A prima facie diagnosis of plague without manifest buboes or manifest lung involvement is not difficult in the ultimate stage of the disease, because it is then possible to detect large or at least appreciable, numbers of plague bacilli in blood smears Earlier in the disease the organisms are few and far between, the clinical signs are only those of a serious general infection and are hence without pathognomonic significance However, as pointed out by Bouffard & Girard,[15] puncture of the liver may be used with advantage at this stage, and it is also noteworthy that Goldstein [48] was successful when puncturing the lungs in cases of pulmonary plague where no manifest signs of pneumonia could be detected Conseil & Durand [27] recommended lung puncture also in cases of pneumonic plague where no sputum was available especially in children who, as a rule, did not expectorate

The laboratory diagnosis of septicaemic plague can be easily established through culture tests or animal experiments with blood specimens taken even early in the disease, but by the time the results of such examinations become available the patients will be beyond hope, if not already dead Their only chance lies in drastic treatment started as soon as the presence of the infection is suspected

Dead bodies

Whenever a complete autopsy is possible, no difficulty will be encountered in establishing a prima facie diagnosis in the case of typical bubonic plague even if the presence of the disease had not been previously suspected, because the characteristic features of a haemorrhagic type of adenitis

are hardly ever found in morbid processes due to other causes (van den Berg & Vos [4])

Cases where the bubonic lesions are not marked may be far more easily overlooked unless the possibility of plague is kept in mind and adequate laboratory examinations are undertaken

The same may be said to hold true of instances of primary respiratory infection where pneumonic changes are slight or even absent Cases of typical primary pneumonic plague usually show such distinctive changes in lungs and pleurae that they should lead if not to a prima facie diagnosis at least to the suspicion that some peculiar infection necessitating careful bacteriological examination is present Still the fact should not be overlooked that certain other infective processes especially influenza may produce gross lung lesions not dissimilar to pneumonic plague and that some instances are on record where micro organisms closely resembling *P pestis* were found in individuals showing pneumonic features at autopsy (Pollitzer [69])

Not only for lack of facilities but mainly on account of popular prejudices it is often impossible to perform complete autopsies in instances where death from plague is suspected Various methods have been recommended to obtain under such circumstances material for laboratory examination

Excision

Some workers such as Lefrou [64] and more recently Macchiavello [71] advised excision of the buboes and or pieces of suitable internal organs (e g the liver the spleen or if pneumonic plague was suspected the lungs) in place of complete autopsies but it must be feared that even this simplified procedure would be much resented in countries where any mutilation of dead bodies is abhorred Serious consideration should be given however to the possibility of using the viscerotome in place of dissection procedures as has been successfully done by plague workers in Ecuador [2]

Puncture

A procedure much used in place of dissection methods for obtaining material for laboratory examinations was puncture of various organs of the dead bodies In the case of bubonic plague the buboes were usually advocated for this purpose but satisfactory results were also obtained both in this form of the disease and in septicaemic plague by puncture of the liver (Bouffard & Girard [15]) Opinions as to what organs should be chosen in the case of pneumonic plague were somewhat divergent: some workers advised puncturing several parts of both lungs as well as the liver

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or heart (Girard,[38, 40] Petrie,[87] Kamal[49]) Such elaborate procedures were recommended because sometimes lung punctures failed when material was taken from non consolidated parts of the organ (Wu Lien teh[111])

Generally speaking, it must be realized that negative results with materials obtained by puncturing the organs of dead bodies do not exclude the possibility that the individuals in question might have succumbed to plague, *particularly if smear examinations alone are used to support the* diagnosis On the other hand, owing to the not infrequent presence in the dead bodies of coliform or other bacilli resembling *P pestis* the exclusive use of smear examination may yield false positive results It should be noted, in this connexion, that Gram negative bacilli of a suggestive appearance are conspicuous in smears made with material from the gastro intestinal tract, which may be punctured in place of the liver or spleen by inexperienced workers However, cells are practically entirely absent in such preparations

A useful procedure recommended by Vincke[11] and Girard[40, 41] for facilitating animal experiments with the materials obtained by puncturing dead bodies was to wash out the syringe, after puncture, with sterile normal saline and to use the fluid for the inoculation of guinea pigs Vincke repeatedly punctured the buboes and/or various organs washing out the needle and syringe after each puncture with the aid of 3 ml of sterile saline which had been placed in a 10 ml flask, and using the resulting suspensions to inoculate guinea pigs cutaneously with the aid of cotton plugs He found that material taken in this way within 24 hours after the death of the victims remained suitable for animal inoculation for 5 days provided that the temperature did not exceed 35°C A similar method was used by Girard who took material from any bubo present as well as from the liver and the upper, middle, and lower parts of both lungs

Bone marrow examination

Several workers obtained satisfactory results when using the bone marrow of plague suspect corpses for laboratory tests Thus Ramos Diaz[91] was able to make a retrospective diagnosis of a pneumonic plague outbreak in Peru by examination of the bone marrow from a rib of one of the victims who had been buried for two months

Some of the workers in South America recommended obtaining bone marrow for laboratory tests from dead bodies by digitomy, i e, amputation of a finger (preferably the most easily amputated forefinger) and opening the second or third phalanx Lobo & Silvetti[69] found this material because scant in plague bacilli, unsuitable for smear examination, but obtained good results by cultivation or animal experiments

Alvarado[1] dealing with the technique of 'digitomy', recommended disinfecting the finger, before amputation with alcohol After the opera

tion which could be safely performed without rubber gloves, the operation wound was covered up with a cotton pad dipped in formalin and fixed with a bandage or the hand was simply placed under the clothing of the dead body For transport to the laboratory the amputated finger was placed stump down in a bottle containing absorbent cotton Extraction of the bone marrow was somewhat difficult but could be facilitated by opening the bones lengthwise instead of crosswise Alvarado suggested that in the plague regions compulsory digitomy should be performed in all cases where death after an acute illness of less than ten days duration had occurred

The method was also recommended by Barreto & Castro [3] who advised using the bone marrow extracted immediately after amputation for the preparation of a roll culture in sulfite agar which was forwarded to the laboratory The same procedure was used to dispatch material obtained through bubo puncture or section of the veins

Useful as these methods of bone marrow extraction are they would one fears meet with much opposition in some of the plague areas It might be possible however under such conditions to obtain bone marrow for postmortem examination from the sternal bone as is done in clinical work It is of importance to note in this connexion that Modica [9] obtained positive cultural results in 10 out of 12 instances with the bone marrow obtained through sternal puncture from plague patients

Preservation of Material

Not only in the case of wild rodent infection but also in that of rat caused plague which shows an increasing tendency to evolve in remote rural localities it has become a matter of great importance to ensure that the materials to be forwarded for examination reach the often distant laboratories in a fit condition

As has been noted some workers instead of relying on methods of preservation preferred to use the material for examination immediately after its collection for the preparation of cultures (see Barreto & Castro [3]) or the inoculation of test animals (see Devignat [5] and van Riel & Mol[9]) but for various reasons it is often impossible to take advantage of these procedures In such cases it is necessary to preserve the vitality and virulence of the materials to be sent to the laboratories either by physical means or by keeping them in fluids or other substances counteracting putrefaction

Into the first mentioned category fall various procedures for keeping the materials for examination at a low temperature pending transport

A method adopted for this purpose in Argentina was to use for the transport of rats large metal drums provided with double walls and to put ice into the outer compartments of these containers which had a capacity

for 20 60 rats[80] Henriques[55] recommended putting the tissues to be forwarded into test-tubes with paraffined stoppers, and enclosing the tubes for transmittal in thermos flasks filled with broken ice A similar method is used in the USA (Link[67]) Macchiavello & Paracampos[72] studying the viability and virulence of *P pestis* in the tropics, noted that if guinea pig or rat organs were kept in the icebox, survival of the plague bacilli was better in the spleen than in the liver in which apparently a rapid autolysis inimical to the micro organisms, took place For this reason some laboratories did not recommend forwarding liver specimens for examination

It is obvious that preservation on ice of plague suspect carcasses or organs pending transit will prove useful only as long as the distances to be covered are not too great or if rapid means of transport are available This is not universally the case and, moreover, in many of the rural plague foci, ice is not available The recent proposal of Amies[2] to use common salt instead of ice deserves, therefore due attention His method consisted of putting the suspect carcasses into a dry jar with a cap which was screwed down tightly after 2 3 ounces (60 80 g) of salt had been added The salt was then made to cover the body of the animal by rotating the jar It was best to examine the bone-marrow of animals preserved in this manner Positive results were obtained in this way even from a plague infected animal kept for 67 days

Broquet advocated the preservation of plague suspect organs in a fluid containing 20% glycerol and 2% calcium carbonate, stating that *P pestis* in organs kept in this manner remained virulent for periods up to 13 days[16] The usefulness of this method was confirmed by several workers Uriarte & Morales Villazon[110] recorded in this connexion that, in the spleens of experimentally infected animals which had been preserved in Broquet s fluid either at room temperature or in the refrigerator plague bacilli usually remained virulent for 12 19 days, sometimes even longer Prado jr[90] found Broquet s fluid useful for forwarding the lymph nodes of plague suspect rats to the laboratory After arrival the tissues were placed on sterile filter paper to remove the excess of glycerol and were then used for cutaneous or subcutaneous inoculation of test animals Issaly & de Issaly[58] recommended the following formula for preparing Broquet s fluid 20 ml of pure neutral glycerol (Baume 30°), 2 g of calcium carbonate and distilled water to 100 ml The fluid was sterilized for 10 minutes at 100°C and then kept in bottles, the corks of which had been sterilized by dry heat and afterwards impregnated with sterilized melted paraffin to prevent development of fungi These authors found that the spleens removed from experimentally infected guinea pigs even two days after death continued to harbour virulent plague bacilli up to 15 days when kept in the fluid They recommended its use for forwarding the organs of plague suspect animals, preferably spleen or bone marrow, to the laboratory To prevent acidification of the fluid which like the formation of fungi biased its efficiency they advised buffering with

bisodium phosphate and citric acid (1 1046 g and 0 0230 g respectively, per 100 ml of fluid)

As established by Devignat[21] and Henriques[5] infection of experimental animals could be produced with plague fleas kept in Broquet s fluid for periods up to 6 days

Satisfactory results were also obtained by using saline solutions in place of Broquet s fluid for the preservation of plague suspect organs or fleas Webster[113] who seems to have been the first to suggest this method recommended that in the case of fresh carcasses a few drops of heart blood be taken with a sterile pipette through a seared portion of the heart surface and added to one ml of sterile citrated saline in a small test tube Such samples were apt to remain suitable for laboratory examination for some days

As established by Girard[39] suspensions in normal saline obtained from experimental animals 3 10 hours after death by puncture and washing out of the syringe remained virulent for up to 6 days when kept at a temperature of 16° 26°C but for 3 days only when kept at 37°C Suspensions from the carcasses of experimental animals which had been kept after death for 48 hours at 21°C remained virulent for 24 hours only but the organs of such animals were still infective after 3 days

Eskey & Haas[33, 34] recommended 2% solutions of sodium chloride for the transport of fleas to the laboratory This concentration while inhibiting the growth of contaminating bacteria was not harmful to the plague bacilli Meyer[5] even used 3% saline for forwarding fleas during summer but took care to wash them in normal saline before grinding them up for animal tests

The methods of preserving plague suspect organs in paraffin with a melting point of 42° 44°C (Henriques[55]) or in mixtures of vaseline oil and vaseline (or lanolin or paraffin) as recommended by Berlin & Bacheva[5] have not been widely used For various reasons Girard[4] considered the latter method of little practical value

Synopsis of Diagnostic and Differential Diagnostic Methods

Laboratory methods for the diagnosis and differential diagnosis of plague have already been discussed in chapters 3 and 4 Nevertheless it seems advisable to summarize them here at the same time indicating their characteristic values

Motility tests

As has been mentioned when dealing with the bacteriology of plague the differential diagnostic value of motility tests is not absolute since *P pestis* is invariably immotile while *P pseudotuberculosis* though typically

motile may occasionally fail to show movement. Generally speaking these tests are, however, of importance, the more so since with their aid it is possible to establish quickly the fact that otherwise suspect strains which show motility are not those of plague.

In place of hanging drop examinations some plague workers recommended the use of Levinthal's[65] method. A useful modification of the latter was described by Himmelfarb:[46]

Small drops of broth cultures of *P. pestis* are placed without spreading on thin agar, the plates are then incubated for 24-48 hours at 20°C. A small coverglass is placed next to a suitable colony and a second coverglass, with one edge resting on the first, is put in a slanting position over the colony. A little saline is placed over the colony with a capillary pipette. Motility is then studied with the aid of a high power dry objective or the immersion lens.

To facilitate a distinction between active Brownian movement and true motility Himmelfarb recommended replacing the saline by a 1‰ solution of mercuric chloride which, while not interfering with Brownian movement, rendered actively moving micro-organisms immotile.

Smears

Fixation. Many workers, both because the usual method of fixation by passing the preparations through the flame does not ensure killing of the plague bacilli and because specimens treated in this way show less clear bipolar staining, prefer fixation of plague smears with alcohol. For this purpose the air dried smears are treated for at least one minute with 95% ethanol, an ethanol ether mixture, or preferably absolute methanol. Unpurified or denatured methanol such as that used as fuel may be substituted. If many specimens have to be handled, it is expedient to immerse them in staining jars filled with alcohol. After fixation the alcohol is poured off and the specimens are dried. Some workers advise the washing off of the alcohol with water before drying the smears in the air or with the aid of blotting paper. If smears have to be sent to a distant laboratory for examination, it is, according to Webster,[113] best to fix them in alcohol for 10 minutes and, after drying, to dispatch them unstained.

Staining. Although different workers recommend various and sometimes rather elaborate methods of staining plague smears, it is as a rule quite satisfactory to use, in addition to Gram's method, the simple stains ordinarily available in laboratories, such as dilute carbolfuchsin, Lœffler's methylene blue, or 1% carbolthionin blue. Should bipolar staining be indistinct, the alcohol fixed specimens may be treated before staining for half a minute with 0.5% acetic acid (Gaffky, quoted by Dieudonné & Otto[8]). Petragnani[86] found it advantageous to use, instead of Lœffler's methylene blue, a stain made up by adding to 10 ml of distilled water 2-3 drops of old saturated methylene blue and 12 drops of a 1‰ solution of lactic acid, staining at slight heat for half an hour.

Gram s method of staining is particularly indicated when dealing with sputum pus from buboes or material from putrified carcasses or dead bodies so as to facilitate the differentiation of the Gram negative plague bacillus from Gram positive micro organisms such as the pneumococcus and certain spore bearers which may resemble *P pestis* in simply stained preparations

Webster[113] advocated using Jensen s modification of Gram s method for plague smears while de Smidt[101] was in favour of the following method

Alcohol fixed smears were stained for 15 seconds in a solution consisting of 1 g crystal violet 10 ml absolute ethanol and 300 ml distilled water after washing the smears were treated for a few seconds with Gram s iodine until brown or iron grey and acetone was then directly applied until no more colour was given off After rapid washing the smears were counterstained with 1% neutral red solution

Evaluation Modern workers are unanimously of the opinion that particularly when dealing with incipient outbreaks or with sporadic instances of the infection smear examinations alone are insufficient to establish the diagnosis of plague

As has been discussed before (page 222) instances of inapparent infection where smears yield a negative result but where animal experiments prove positive are frequently observed in rodents Similarly culture tests or animal experiments may sometimes establish the diagnosis of human plague in instances where smear examinations gave a negative result The presence of morphologically atypical plague bacilli may also confound the issue when sole reliance is being placed on an inspection of smears

On the other hand examination of smears is often apt to suggest the presence of plague in instances where the infection is absent Pasteurellae other than the plague bacillus showing morphological features similar to or even identical with those of the latter organism have often been found in rodents and occasionally also in man Moreover in decomposed carcasses or dead bodies bacilli belonging to other genera may show microscopic appearances similar to those of the pasteurellae and as was claimed by some observers e g de Smidt[101] and Hennessey[51] the presence of such organisms may prove misleading when sputum smears of patients suspected to be suffering from pneumonic plague are examined

The limitations of smear examination are well illustrated by the following results obtained by Girard[45] through the combined use of this method and of animal experiments in the case of materials which had been collected from human plague victims with the aid of punctures

Smears	Total number of combined examinations	Animal experiments Positive	Negative	Positive inoculations (%)
Positive	781	690	91	88 3
Suspicious	962	409	553	42 5
Negative	2 642	124	2 518	4 7

In spite of these shortcomings a judicious use of smear examinations is indispensable in order to watch the trend of fully confirmed epizootics Smear examinations are also of some importance for the recognition of human plague particularly in clinically obscure cases of bubonic plague

Cultures

The methods of cultivation which appear to be of prime importance for the identification of *P pestis* and for its differentiation from other bacterial species may be grouped according to whether they are intended to take account of

(1) morphological and growth characters,
(2) growth requirements,
(3) selective growth requirements

Characteristics of morphology and growth (*a*) *Involution forms* It is of importance to note that, as first shown by Hankin & Leuman cultivation of the plague bacillus on agar containing 3% sodium chloride leads to the production of marked involution forms True enough claims that this phenomenon is specific for the plague bacillus to the exclusion of *P pseudotuberculosis* have not been substantiated However the morphological features noticeable when the latter is grown on 3% salt agar are apt to be different from those manifest in the case of the plague bacillus

Thus Topping et al,[107] growing a pseudotuberculosis strain of human origin on 3% salt agar, noted the presence of longer rods and of coccoid and some swollen forms but stated that 'it was not the characteristic pleomorphism of plague Likewise Haas,[51] cultivating a pseudotuberculosis strain of rat origin on 3% salt agar, obtained involution forms which were " usually long, slender slightly curved rods or medium sized rods lying in bundles in a manner suggestive of the diphtheria bacillus less frequently there were enlarged hollow staining organisms resembling balloon forms of involuted *P pestis*

It moreover appears that, as first noted by Zlatogoroff (quoted by Dieudonne & Otto [28]) and confirmed by other observers [13] involution of plague bacilli on 3% salt agar becomes manifest in 24 hours—that is more rapidly than in the case of other bacterial species

(*b*) *Stalactite' growth* The peculiar crumbly and stalactite growth of *P pestis* in broth can no longer be considered of differential diagnostic importance because such features, while not invariably present if this micro organism is cultivated may be produced by the rough form of the pseudotuberculosis bacillus Broth cultivation of an otherwise identified plague strain is however of value in so far as absence of turbidity renders it likely to be a pure culture of the organism

Requirements for growth (*a*) *Plain agar* In order to distinguish between plague and pseudotuberculosis bacilli advantage may be taken of

the fact that the former generally grows sparsely on plain agar while the latter develops abundantly after 24 hours incubation (Bezsonova et al [8]) To carry out such tests care must be taken to use inocula sufficient in size to permit growth of the plague bacillus On the other hand, it must be kept in mind that if material for cultivation is taken directly from the animal or human body admixture of blood or tissue fluids may lead to an unusually abundant growth of *P pestis* on plain agar

(*b*) *Small inocula* Among the numerous methods which have been utilized to induce growth of *P pestis* from small inocula the following simple procedure recommended in the *Report of the Haffkine Institute for the year 1931* [105] is fully satisfactory for diagnostic and differential diagnostic purposes

With the aid of a 1 mm loop dilutions of the micro organisms to be tested are made in tubes each containing 10 ml of normal saline Blood agar slants are then inoculated with 1 mm loopfuls of the dilutions and plain agar slants with 5 mm loopfuls In the case of the plague bacillus discrete colonies appear on the blood agar slopes and none at all on plain agar while pseudotuberculosis bacilli develop equally well on both media

(*c*) *Exhausted media* Basing his recommendation on earlier work by Fabiani [35] Petragnani [86] advocated for the differentiation of plague bacilli from other bacterial species the use of old culture media on which plague bacilli had been grown and which had been sterilized after the requisite amount of water had been added Plague bacilli failed to develop on such " exhausted media while other micro organisms developed freely

(*d*) *Hungry media* Bezsonova [6] (see also Bezsonova et al [7]) found hungry agar prepared without peptone suitable for differential diagnostic work pseudotuberculosis bacilli developed fairly well on such media while plague bacilli grew poorly if at all

(*e*) *Bile media* It should be added that bile media although not suitable for differentiating the plague from the pseudotuberculosis bacillus have been used with advantage for the enrichment of primary blood cultures Kirschner [60] found pure bile previously recommended by La Rosa [6] sterilized either by filtration or by heating for 20 minutes at 110°C satisfactory for preliminary cultivation of blood or pus By this method good growth could be obtained with small inocula (10 40 plague bacilli per ml) in fact even the addition of one drop of blood to 5 ml of bile was sufficient to show up a slight bacteraemia Ohoto [83] [84] first advocated the use of Conradi and Kayser s bile medium for taking blood cultures in plague work but afterwards used a medium consisting of 500 ml of fresh ox bile to which 5 g of peptone had been added This was steam sterilized for two hours divided into 50 test tubes and again heated for one hour

For routine work the simple procedure recommended by Sokhey & Wagle [103] is however fully satisfactory This consisted of using 0 5 ml of

blood, obtaıned from a veın of the patıent, to ınoculate two agar slants (0 25 ml per slant) which were then ıncubated for two days at room temperature

Requırements for selectıve growth (*a*) *Low temperatures* In order to ısolate plague bacıllı from contamınated materıals advantage has been taken of the abılıty of thıs mıcro organısm to grow at low temperatures Petra gnanı [86] recommended for thıs purpose ıncubatıon at 10°C but, as has been noted when dealıng wıth the growth of *P pestıs* on gelatın, even freezıng of medıa ınoculated wıth contamınated materıal has been employed

(*b*) *Medıa counteractıng contamınants* Besıdes the above mentıoned method, cultıvatıon on medıa able to counteract the growth of contamı nants was recommended for work wıth such materıals Drennan & Teague [79] advocated an agar medıum prepared from beef heart to which 0 025% sodıum sulfite and 0 00143% of crystal vıolet had been added Sımılarly Meyer and Batchelder [76] recommended addıtıon of 0 025% sodıum sulfite and 0 0025% gentıan vıolet to hormone beef heart agar Kıster [61] found Endo s medıum useful for work wıth putrefied rats sınce ıt restrıcted the growth of *Proteus vulgarıs* However ıt was necessary to establısh empırı cally the amounts of sodıum sulfite and of fuchsın most favourable for thıs purpose The use of the two medıa mentıoned above ıs therefore more expedıent

Bıochemıcal reactıons

Carbohydrate substances (*a*) *Technıques* As dıscussed by Pollıtzer,[89] solıd medıa have been used to study the actıon of *P pestıs* on carbohydrate substances, and Francıs [37] obtaıned good results wıth the semı solıd medıum of Enlows [31] , lıquıd medıa have, however been utılızed for such tests by most recent workers, and seem to be preferable The technıque adopted by Chen [0] on the advıce of K F Meyer and Pollıtzer was as follows 1% peptone water (prepared wıth a brand of peptone not contaınıng carbo hydrate lıke fractıons which mıght gıve false posıtıve results) served as base to which the fermentable substances were added ın 1% proportıons Andrade s ındıcator was ıncorporated and the final pH of the medıum was adjusted to 7 4 The cultures to be tested were first streaked out on blood agar plates to check for contamınatıon If found pure, loopfuls of the growths were transferred ınto the approprıate medıa The tubes were then ıncubated at 37°C As recommended by de Smıdt,[100] the growths were aerated by shakıng the tubes daıly Readıngs were taken daıly for a perıod of 21 days In order to make sure that thıs procedure kept the bacıllı alıve subcultures on blood agar plates were made once weekly from each tube under test

Another possıble source of error to be guarded agaınst ın condıtıons prevaılıng ın the tropıcs ıs the spontaneous hydrolysıs of saccharose

constant control with Fehling s solution is therefore necessary (Devignat & Boivin)

In their recent studies Devignat & Boivin took advantage of the micro glucide dish devised by the former author [6] To utilize this expedient and economical method Devignat & Boivin first grew the plague strains to be tested for 48 hours at 30°C in 12 ml of peptone water containing Andrade s indicator 0 5 ml quantities of these growths were then placed in the tubes of the dish each of which contained 0 5 ml of one of the carbohydrates to be used in a 4% solution made with twice distilled water An observation period of 8 days was found sufficient when applying this method

In addition to the tests described above a simple and expedient method devised by Uriarte & Morales Villazon [109] deserves mention These workers used for their test 2% peptone water slightly alkaline to litmus with 8 g of glucose or laevulose and 4 6 ml of a 1% aqueous neutral red solution per litre After sterilization at 110°C this was poured into Durham tubes Uriarte & Morales Villazon claimed that growth of plague bacilli in this medium produced a characteristic triad of signs (*a*) slight acidity with a corresponding colour change to yellow (*b*) absence of gas production (*c*) formation of a flocculent precipitate which settled down so that the fluid remained clear They claimed that none of the micro organisms likely to be confused with the plague bacillus showed this combination of reactions

Devignat & Boivin [27] while confirming these results in general found in three out of 36 *instances slight turbidity instead of formation of a flocculent deposit*

Another simple method recommended by Webster [113] involved the use of 1% peptone water containing glucose mannite lactose and saccharose *respectively besides an indicator After incubation at 37°C for* 48 hours there should be production of acid but not of gas in the Durham tubes containing glucose and mannite and no change in those containing lactose and saccharose

(*b*) *Evaluation* As has been discussed previously the claim made by earlier observers that tests with glycerol containing media were of value for a differentiation between plague and pseudotuberculosis bacilli can no longer be considered as valid The importance of tests with rhamnose containing media is still stressed but it must be noted that (*a*) according to Russian observers glycerol positive plague strains isolated in south east Russia and the interior of Asia were apt to dissociate into rhamnose acidifying and rhamnose negative variants (*b*) late rhamnose acidification produced by plague strains has been observed and (*c*) Devignat & Boivin [7] working with 40 strains isolated in the Belgian Congo observed slight rhamnose acidification in one instance It would be unwise therefore to deny the plague character of an otherwise suspect strain merely because it shows activity towards rhamnose media At the same time however

exceptions of this kind do not invalidate the rule that in contrast to pseudo tuberculosis bacilli, plague bacilli (as well as the pasteurellae in the strict sense) produce no acidity in rhamnose media

Hydrogen sulfide and indole Tests for the presence of hydrogen sulfide and indole are of limited differential diagnostic importance, because both plague and pseudotuberculosis bacilli fail to produce these substances in the course of cultivation A simple test devised by Webster [113] is as follows A tube containing 1% peptone water should be inoculated with the bacilli to be tested and a few drops of 5% lead acetate solution put on the inner end of the plug which should be made of white cotton wool If hydrogen sulfide is absent, the plug should show no blackening after incubation at 37°C for 24 hours To the inner end of the plug of the same culture (covering the plug if necessary with a new layer of white cotton wool) a drop of 1% potassium persulfate and then a drop of Ehrlich s rosindole reagent [c] should be added If indole is absent, there should be no pink colour on the plug after incubation for 24 hours at 37°C

Milk Tests with litmus milk (see also chapter 2) cannot be considered as fully reliable for a differentiation of the plague from the pseudotuber culosis bacillus because some strains of the latter species are poor alkali producers Results obtained through cultivation of the plague bacillus in litmus milk are also somewhat inconsistent, Devignat & Boivin [27] for instance, finding that the medium remained unchanged in 33 out of 38 strains tested while slight acidity was produced 4 times and slight alkalinity once

Nitrates As discussed in chapter 3, procedures demonstrating respec tively a reduction of nitrates to nitrites and production of nitrous acid are not universally valuable for a differentiation of plague and pseudo tuberculosis bacilli, even in areas where positive reactions were usually given by *P pestis*, occasional negative results were noted Webster s [113] simple reaction test is as follows A 5 mm loopful of the same culture recommended for his hydrogen sulfide test is placed on a white opal glass plate and mixed with a 2 mm loopful of Ilosvay s reagent [d] If nitrites are present a pink colour should appear within a minute

Attention has been drawn to the potential value of Fauconnier s [38] proposal to differentiate between plague and pseudotuberculosis bacilli with the aid of tests showing the urease activity of these organisms How ever the favourable results reported by this author require verification

[c] Ehrlich s rosindole reagent is prepared by taking 1 g of p dimethylamino-benzaldehyde 95 ml of absolute ethanol and 20 ml of concentrated hydrochloric acid and mixing the whole with an equal quantity of rectified spirit

[d] Ilosvay s reagent is prepared by mixing equal parts of (a) 1 g of sulfanilic acid 14 7 ml of glacial acetic acid 285 ml of distilled water and (b) 0 2 g of naphthylamine 14 7 ml of glacial acetic acid and 3 5 ml of distilled water

Serodiagnostic tests

As has been stated previously when dealing with the problem of sero diagnosis advantage should be taken of agglutination to test unknown strains with plague immune sera of established potency, and to examine on the other hand the sera of suspect patients with the aid of known cultures

While satisfactory procedures including slide tests convenient for the rapid diagnosis of human plague have been worked out, the problem of to what extent agglutination may be relied upon for the identification of unknown strains and consequently for a differentiation of plague and pseudotuberculosis bacilli, is not yet solved The methods proposed by Bhatnagar[8] and by Seal[9] appear to be promising but their differential diagnostic value should be confirmed by large scale investigation

In addition to the methods of agglutination haemagglutination and complement fixation dealt with in chapter 3 the following serological tests seem to possess practical value for the diagnosis particularly the retrospec tive diagnosis of human plague (*a*) the flocculation test introduced by Girard[43] (see chapter 3) using the serum of patients or convalescents and toxic filtrates or extracts of *P pestis* and (*b*) the allergic reaction recommended by da Silva jr & de Albuquerque[99] (see also da Silva jr[97]) These authors used an antigen prepared by removing the bubo of a sub cutaneously plague infected guinea pig immediately after death boiling this material for two hours in normal saline grinding it in a sterile mortar at the proportion of 1 g per 20 ml of saline filtering through sterile gauze and finally adding 0 5%$_0$ phenol If found sterile this material was used in quantities of 0 1 ml for intradermal injection A control injection with an equal amount of milk was made 4 cm from the site of the antigen injection Readings were taken after 24 36 and 48 hours In positive cases a papule surrounded by an inflammatory zone developed the reaction becoming maximal after 36 hours

Some workers such as Cambosu[17] Menezes[71] and Tumansky[108] recommended the use of serological methods for the rapid diagnosis of rodent plague A further precipitin test was recently recommended by Larson et al[63] These workers established by preliminary investigations that boiling of the materials to be tested as formerly employed in carrying out thermoprecipitin tests (Piras and others quoted by Pollitzer[89]) led to a decrease in the antigen content Diethyl ether was therefore used to sterilize the culture or tissue suspensions to be tested with plague immune sera Precipitin tests performed with antigens obtained in this way from tissues of animals which had died of plague showed the presence of soluble antigens in sufficient quantities to be of diagnostic value in instances where the material had been stored at 37°C for periods of at least 14 weeks

In places where adequate laboratory facilities are available complement fixation tests carried out according to the method of Chen et al[1] (see chapter 3 p 169) may prove valuable for the diagnosis of rodent plague

acteriophage tests

As discussed when dealing with the bacteriophage problem Gunnison al[50] recently found that tests carried out with plague phage at 20°C fered a means of differentiating between plague and pseudotuberculosis cilli

To facilitate the application of such tests, Cavanaugh & Quan[18] soaked rilized strips of filter paper in broth cultures of *P pestis* which had been ded with a potent plague phage and then subjected the strips to lyophiliza n or desiccation in vacuo If such strips were applied to freshly made gue subcultures on blood agar plates and these were incubated at C, a 1-mm wide zone of lysis usually became visible in 18 24 hours nd the bacteriophage coated strips while no such clearance was produced nd noncoated strips of filter paper used as controls Tests made under itical conditions with growths of *P pseudotuberculosis* gave negative ilts As far as established up to now, the bacteriophage coated strips erved by lyophilization remained capable of producing lysis for periods hree months if sealed under nitrogen and stored at room temperature iccated strips did not display such keeping qualities

Should the value of this procedure be confirmed by extensive trials it ld form an easy means of differentiating plague and pseudo rculosis bacilli, the more so as it might be possible to supply field kers or persons operating in areas without facilities for bacteriophage , with test strips prepared in central institutions

ial experiments

1 evaluating the methods available for the laboratory diagnosis of it plague stress has been laid on the importance of animal experiments rmed with pooled organs or pooled fleas At the same time however s pointed out that invaluable though these methods are for giving overall picture of the situation they fail to yield information on the t and degree to which plague is present Other methods for filling gap must therefore be used side by side with pooling experiments y to carry out animal experiments with material from individual ed rodents would not be sufficient to establish to what degree they een infected, and would, moreover, be quite out of question in the of considerable epizootics However, such experiments are called ien dealing with initial or sporadic manifestations of rodent plague e position in regard to human plague is similar Here also it is al to verify the diagnosis in initial or sporadic cases by all available ds including animal experiments to continue these with each patient a considerable epidemic would however be impossible

choice of experimental animals to be used for plague diagnosis ls upon the nature of the work to be performed Generally speaking,

guinea pigs should be chosen for pooling tests and these animals are also most suitable for confirming the diagnosis in initial or sporadic plague manifestations As recommended by Lloyd[69] three guinea pigs, infected respectively through the unbroken skin, subcutaneously and intraperitoneally should be used when dealing with initial or sporadic manifestations of the infection While the most rapid results may be expected from the intraperitoneally infected animal the likelihood of obtaining a pure culture is greatest in the case of the percutaneously infected one In tropical climates particularly it is advisable to kill moribund animals (i e those which lie down on their sides) with the aid of chloroform because this procedure besides saving time increases the chances of isolating pure growths

Intraperitoneal infection of white mice is a method of unsurpassed value for establishing the existence of a bacteraemia and at the same time confirming the diagnosis in instances of human plague Moreover it will often be possible to use these animals which can be easily bred and kept at small expense on a larger scale than guinea pigs A drawback is that white mice are far more susceptible than guinea pigs to pneumococcus infections (Girard[44])

White rats are of outstanding value for differentiation between plague and pseudotuberculosis bacilli It is true that a few strains of the latter species have been found pathogenic for white rats but such aberrant results may also be obtained when using most of the other methods available When performing differentiation tests the cumulative evidence obtained in various ways rather than the results of any individual method should be taken into account (Schutze[91])

Precautions Advisable

The dangers confronting plague laboratory workers are threefold

(1) the possibility of contracting bubonic infection through the bite of blood sucking insects especially rodent fleas

(2) the possibility of contracting direct bubonic infection when performing postmortems or otherwise handling contaminated material

(3) the possibility of contracting pneumonic infection through splashing or spraying material laden with plague bacilli

Bubonic infection through fleas

When it is necessary to enter plague infected houses or when coming into contact with persons or dead bodies or with rodents possibly harbouring infected fleas it is safest to wear a special costume consisting principally of a gown similar to that used by clowns which made of one piece covers

the whole body except the head and hands This is donned through the opening in the neck and is then tied firmly round neck and wrists The costume is completed by rubber or high leather boots a linen cap and as far as necessary, by rubber, canvas, or leather gloves A mask must be added if there is danger of pneumonic infection

Since it is rather trying to wear the above mentioned gown under tropical conditions, long linen stockings covering the feet and legs and tied up above the knees may be substituted, or high boots (made from rubber or leather) into which the trousers are tucked, alone, may be used

It has been recently recommended that protection against fleas should be ensured by impregnation of the underwear with DDT or impregnation of the clothes with other modern insecticides (Elishewitz [30] Linduska et al [66] Smith & Burnett[10]) A combination of such applications with the use of long linen stockings and/or high boots would seem particularly effective

Safe methods for handling rodents delivered to the laboratory for examination and for freeing them from fleas have been described earlier (see pages 225, 226) Experimental animals kept in the laboratory should be housed in rat proof rooms or at least in rat proof cages To protect infected animals against stray fleas, the containers accommodating them may be covered with gauze, surrounded by tanglefoot paper, or suspended

Direct bubonic or pneumonic infection

If one works with the cleanliness and carefulness indispensable for laboratory work in general no special precautions need be observed when examining plague material by bacteriological methods To minimize the danger of accidents, glassware of the best quality should be used exclusively for plague laboratory work Slides should be immersed immediately after examination in a jar containing an antiseptic fluid (preferably industrial spirit) and should be boiled before cleaning

When performing human autopsies a proper costume should be worn, including a rubber apron and high rubber boots Solid rubber gloves are indispensable In his autopsy work, done before sulfonamides and antibiotics had become available for treatment the present writer used two pairs of rubber gloves, the cuffs of the gown being tied down over an inner pair of the usual medium weight pattern, and hands and forearms then being covered by a long pair of solid postmortem gloves

To avoid danger from occasional splashing masks and preferably also goggles should be worn

Less stringent precautions are necessary when dissecting plague infected animals the more so because plague workers should learn to perform autopsies with the aid of suitably long instruments without touching the carcasses However, even if this is done rubber gloves should be used as a precaution against accidental touching of the animals or splashing of infectious material

The carcasses of the dissected animals should be burned immediately after autopsy together with the contents of the containers where the animals have been confined Cremation is also preferable in the case of dissected human plague victims If this is impossible the dead bodies should be wrapped in shrouds soaked in strong antiseptic solutions before being put into leak proof coffins or preferably quicklime should be used to cover them

Experimental infection of test animals should be carried out with the precautions recommended above for dissection When using injection methods care must be taken to avoid the danger of spraying about infectious material it is best to wear masks whenever infecting test animals with the aid of syringes

All possible care must be taken when manufacturing vaccines or sera with virulent plague strains and when carrying out experimental research work because as shown by several deplorable incidents it is in the course of such activities that pneumonic infections are most likely to occur

A strict supervision of the assistant and lower staff employed in plague laboratories is essential to make sure that they are not remiss in adopting all necessary precautions

If in the course of plague laboratory work any mishap occurs adequate methods of disinfection of the clothes and persons of the workers concerned as well as of the whole room should be started at once Any wounds on fingers or hands whether pre existent or contracted during the accident should be attended to first preferably by thorough soaking in alcohol

If the risk of infection of workers involved in laboratory accidents seems appreciable prophylactic administration of sulfonamides should be resorted to

It is on the other hand necessary to make sure that laboratory workers in order to protect themselves against imaginary dangers do not use sulfonamides indiscriminately Pollitzer saw an instance of serious kidney affection (haematuria and oliguria) in a technician detached for field work who with the idea of protecting himself against plague took 1 2 g of sulfadiazine daily for several weeks Fortunately no permanent harm resulted

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Chapter 6

HOSTS OF THE INFECTION

RODENTS AND LAGOMORPHA

Reviewing in 1928 the then rather limited knowledge available concerning the occurrence and importance of plague in rodents other than the common rats and mice Jorge[89] felt justified in drawing a clear cut distinction between the pandemic type of plague introduced into human settlements and houses all over the world by the domestic rats and mice and peste selvatique which is dangerous for man only when he invades the remote endemic foci populated by wild rodents

Although Jorge s concept was accepted some discussion arose regarding the appropriateness of the term peste selvatique or as Stallybrass[19] and Wu Lien teh[15] translated it selvatic plague It was pointed out by Meyer[1 8] that on etymological grounds the name sylvatic plague would be preferable and this term was widely used until Pozzo[1 3] and Hoekenga[74] doubted and Girard[64] denied its adequacy on the grounds that the word sylvatic implied that the rodents concerned lived in forests whereas that was rarely the case Girard therefore advocated the reversion to the expression wild rodent plague which was used before the publication of Jorge s study—a proposal it has seemed advisable to accept for this monograph[a]

Much more important than the difficulty of adopting an adequate nomenclature is that of distinguishing between rat and wild rodent plague—a distinction which is no longer as clear cut as Jorge was entitled to assume Since 1928 the list of rodents other than the commensal rats and mice known to suffer from natural plague has grown incessantly so that instead of little more than a score almost 200 species or subspecies are now known to be implicated Besides varying in their etiological importance they

[a] It is important to note however that—as has been pointed out by an erudite reviewer of the above passage—the Latin name silvaticus when applied to animals actually means wild rather than forestal and the names derived from it in Spanish Italian and Portuguese convey the same meaning so that Jorge s nomenclature is fully justified on etymological grounds though as it is likely to mislead English and French readers it may be preferable to use the name wild rodent plague

show marked differences in their ecology, many live near to man rather than in the remote haunts of sylvatic plague and thus become apt to take part in the perpetuation and spread of the infection in settlements and even houses. It follows that it is no longer possible to deal merely with wild rodent and rat plague as was done in the past, attention must also be paid to those species other than the commensal rats and mice which, on account of their peri domestic or semi domestic habits, take an intermediate position and are, in part, responsible for a transition of the infection from the wild to the commensal rodents, or vice versa.

Wild Rodents [b]

When trying to trace the gradual evolution of present day knowledge on the existence and importance of wild rodent plague, it is not surprising to find the earliest evidence in Central Asia where the infection probably originated and where it has certainly existed since time immemorial.

As first stated by laymen such as Tsherkasoff—in his *Memories of a hunter in Siberia* (1856 63)—in the case of Transbaikalia and Outer Mongolia, and Prjevalski[160] for the Ordos country of Inner Mongolia and later confirmed by medical men, e g, Rudenko[1] ' Skchivan,[187] and Barykin[14] the inhabitants of these areas had been aware for generations of the periodical occurrence of a fatal, infectious disease in the tarabagans (Siberian marmots) which was apt to spread to man, and had taken surprisingly adequate measures to protect themselves against this danger. The mysterious illness of the marmots was not only the subject of numerous legends but was also referred to in the old Tibetan sacred books. In the opinion of the local practitioners, small worms invisible to the naked eye caused this and other infectious diseases, a claim curiously similar to that arrived at in 1658 by Athanasius Kircher[94] with regard to human plague.

The infection prevailing among the tarabagans, and among the human beings who had come in contact with them seems to have been first identified with plague in 1895 by Bjeliavski & Rjeshetnikoff,[17] probably because their attention had been attracted to this disease by its spectacular appearance at Canton and Hong Kong in 1894. However, the evidence brought forward by these two observers was not supported by laboratory examinations and it was not until 1905 that bacteriological proof of the existence of human plague in Transbaikalia was obtained.[15] Two years later a tarabagan dissected by Barykin[14] was also found positive but it was only in 1921 that the existence of widespread epizootics among these animals was confirmed (Sukneff and others quoted by Wu Lien teh[1]).

These records leave little room for doubt that the foci of wild rodent plague in Central Asia belong to the category of natural foci defined

[b] As discussed later species belonging to the orders of Lagomorpha and Rodentia are involved in hat for the sake of brevity is commonly called "wild rodent plague"

by Pavlovski [150] as arising and continuing to exist independently of factors connected with the presence of man

Whether any other foci of wild rodent plague fall into the same category or became so established long ago is a difficult question to answer The foci in south east Russia and in Kurdistan deserve consideration in this respect but too little is known of their early history to permit of any definite conclusion It should be noted in this connexion that, though wild rodents were occasionally suspected of playing a part in the spread of plague in south east Russia during the first decade of the present century or perhaps even since the Vetlianka outbreak in 1878 9, it was not until 1912 and 1913 that the existence of natural plague in the sisels (susliks) was bacteriologically confirmed by Deminski [41] and by Berdnikov [15] respectively Proof of the existence of the infection among the gerbils (*Meriones*) of Iranian Kurdistan has been obtained quite recently by Baltazard et al [4]

As mentioned in chapter 1 claims have been made that the origin of wild rodent plague in the western parts of the USA was due not to a recent importation of the infection by the sea route but to an early immigration from Central Asia of certain of the species involved however as was pointed out the evidence supporting the latter assumption is not convincing There is no doubt that the foci of wild rodent plague existing in South Africa and in parts of South America became established through a spread of the infection from the commensal to the free living species during the present century

It is of historical interest to add that as far as can be established the first authentic record of natural plague in wild rodents was made by Simond [185] who in 1898 reported positive findings in Indian palm squirrels (*Funambulus palmarum*) at Karachi As recorded by Bruce Low [110] in the following year several porcupines together with some monkeys died of plague during an outbreak at Mysore However, since these animals had been kept in a zoological garden and no other records on porcupines are available one cannot justifiably include them in the lists of rodents other than the commensal rats and mice in which the existence of natural plague has been confirmed or suspected

Species involved

In order to show the occurrence of natural plague in rodent species or subspecies other than the commensal rats and mice and in Lagomorpha lists enumerating (*a*) the animals in respect of which positive proof of the infection has been obtained and (*b*) the suspected animals are contained in Annex 1 tables I and II (pp 623 and 633)

Particular attention has been paid to make Annex 1 not only as accurate as possible by closely following the standard nomenclature of Ellerman [5] and of Ellerman & Morrison Scott [6] respectively but also as complete as possible though it is realized that entire success has not been obtained in

the latter respect Indeed, it seems doubtful whether it will ever be possible to compile a really satisfactory list of plague affected rodents and Lagomorpha other than the commensal rats and mice because it is most probable that, in addition to the known foci, unrecognized foci of sylvatic plague exist, and because even in the areas where the presence of this type of the infection has been ascertained, by no means all the species or subspecies involved have been detected In chapter 1 attention was drawn to the statement of Davis that over 100 rodents or other small animals are at risk of infection in South Africa, and it is also noteworthy that Pozzo[17] believed that 70 rodent species were involved in Argentina

TABLE XIV FAMILIES AND SUBFAMILIES OF RODENTIA AND LAGOMORPHA IN WHICH THE PRESENCE OF NATURAL PLAGUE HAS BEEN CONFIRMED

Family	Subfamily	Number of species or sub species found infected	Area
Bathyergidae	—	1	Angola
Caviidae	Caviinae	9	Argentina Brazil Ecuador Peru
Chinchillidae	—	1	Argentina
Dipodidae	Dipodinae	5	Iranian Kurdistan south east Russia Transbaikalia
Echimyidae	Echimyinae	1	Brazil
Geomyidae	—	2	USA (western States)
Heteromyidae	Dipodomyinae	3	USA (western States)
	Heteromyinae	1	Venezuela
Muridae	Cricetinae	43	Argentina Bolivia Brazil Ecuador Louisiana (USA) Peru south east Russia South Africa USA (western States) Venezuela
	Dendromyinae	4	Belgian Congo South Africa
	Gerbillinae	12	Belgian Congo India Iranian Kurdistan south east Russia Russian Turkestan South Africa Transcaspia
	Microtinae	12	Iranian Kurdistan south east Russia Transbaikalia USA (western States)
	Murinae	30	Belgian Congo Burma Ceylon East Africa Egypt Gold Coast India Kenya Senegal South Africa
	Otomyinae	6	Belgian Congo East Africa Kenya South Africa
Pedetidae	—	1	South Africa
Sciuridae	—	49	Canada Ceylon Ecuador India Manchuria Mongolia Peru south east Russia Russian Turkestan Senegal South Africa USA (western States, Transbaikalia
Leporidae	—	14	Argentina Bolivia Brazil England Ecuador Peru South Africa Transcaspia USA (western States)
Total		193	

Natural plague has been confirmed in the families and subfamilies of the orders of Rodentia and Lagomorpha listed in table XIV

In order to deal adequately with wild rodent plague in the strict sense table XV has been prepared to show (*a*) the reservoir hosts in the definitely established foci where this type of the infection is known to be or to have been, active (*b*) other families or subfamilies, species or subspecies of which have also been found infected and which are therefore apt to play a subsidiary role and, if living near man, are able to act as intermediaries between the strictly wild and the commensal rodents

The data of table XV well bear out the statement of Meyer [130] that

"as a rule a principal species belonging to the family [of] Sciuridae or [to the sub family of] Gerbillinae living in subterranean colonies in families or singly presides over the exchanges of the plague bacillus

A study of the relative distribution of these two main groups of reservoir hosts in the wild rodent foci in or near Asia clearly shows that three zones may be distinguished

TABLE XV LIST OF DEFINITELY ESTABLISHED WILD RODENT PLAGUE FOCI SHOWING RESERVOIR HOSTS AND OTHER FAMILIES OR SUBFAMILIES FOUND NATURALLY INFECTED

Plague focus	Main reservoir	Also found infected
Argentina	Cav inae ([illegible] etc)	Chinchillidae Leporidae
Iranian Kurd t n	Gerbillinae *M [illegible] ie*	Dipodinae Microtinae
Manchuria Mongolia and Transbaikalia	Sciuridae *Ma mola*	Dipodinae Microtinae
Peru	Cricetinae *(ra m)* Cricetina *Ah d [illegible]*	Cav nae Leporidae
Peru Ecuador border region	Sciuridae (*S [illegible] iurus*	Cricetinae
Russian Turkestan	Sciuridae (*Marm [illegible]*	Gerbillinae
South Africa	Gerbillina *Tale a*	Cricetina Dendromyinae Leporidae Murinae Otomyinae P det dae Sciuridae
South-e t Russia northern foci	Sciuridae (*[illegible] llu*	Dipodinae Microtinae
outhern foci	Gerbillinae *M r[illegible] e*	
Transcaspia	Gerbillinae *Me [illegible]*	Leporidae
USA western States Arizona and New Mexico	Sciuridae ([illegible]	
coastal regions and northern part of intermountain plateau	Sciuridae *Cit llu*)	D d m na G m a
southern deserts	Cricetinae *Ne [illegible]*	Le [illegible]
Washington	Microtinae *I [illegible] u*	Cr na D m [illegible]
V n ucla	H t romyinae *Het* Cr etna *S[illegible]*	—

See Link

(1) Central Asia, where large Sciuridae (marmots) form the plague reservoir (Manchuria, Mongolia, Russian Turkestan, and Transbaikalia and perhaps other adjacent areas mentioned in Annex 1, table II (p 633))

(2) South east Russia northern part, where small Sciuridae (sisels) are of prime importance ,

(3) South east Russia southern part, Iranian Kurdistan and Transcaspia where Gerbillinae (*Meriones*) are the reservoir hosts

This separation into a north eastern area, where Sciuridae are the *fons et origo mali*, and a south eastern area where a corresponding role is played by the *Meriones*, is a matter of great interest

Sciuridae (ground squirrels and prairie dogs) also form reservoirs in some of the western States of the USA and in the focus situated at the border between Peru and Ecuador, while Gerbillinae are of paramount importance in South Africa

The Cricetinae form reservoirs in three wild rodent foci—namely Argentina, the Huancabamba area of the Peruvian Andes (Macchiavello[119, 120]), and the southern deserts of some western States of the USA—while the Caviinae, Heteromyinae and Murinae each act correspondingly in only one area The comparatively inconspicuous part played in wild rodent plague by the Murinae to which subfamily the commensal rats and mice also belong seems striking when the ominous role played by the latter species in the pandemic type of plague is given consideration However as will be discussed later the observations made in respect of the multimammate rat *Rattus natalensis* prove that this rule is by no means without exceptions

Characteristics of principally involved species

A short description of the species mainly involved in wild rodent plague and of those of their habits which are of importance in the perpetuation and spread of the infection may be given thus

1 The marmots (tarabagans) of Central Asia and the adjacent plague areas are big animals attaining a length of about half a metre and a weight of 4 to 7 5 kg They dig deep burrows in firm ground and live in settlements of varying size each family inhabiting a separate burrow Following hibernation which lasts from about October to April, they mate in spring After a pregnancy lasting 6 weeks the female gives birth to 2 to 7 young[215] As far as can be ascertained, the young continue to stay in the maternal burrow even after they have become independent while the mother seeks new quarters for herself

2 The small sisels or susliks (*Citellus pygmaeus*) of south east Russia look like miniature tarabagans and have a body weight of 60 to 200 g They use two kinds of burrows shallow ones during the summer and deeper ones in the winter Their hibernation lasts longer than that of the

tarabagan the adults beginning to sleep in July, the young animals in August or September The mating period begins immediately after the end of the hibernation period in early spring After a pregnancy lasting probably not longer than three weeks litters of 8 to 10 young are born

The young animals leave their mothers about a month after birth each settling down in an individual burrow, preferably a previously used one The sisels are therefore most active

(*a*) in early spring when the animals which otherwise lead an individualistic life meet to mate and when the females prepare the burrows for their litters

(*b*) in early or late June according to the weather when the young disperse sometimes travelling as far as 2 to 5 km before settling down

(*c*) a month later when the winter burrows are prepared and occupied (Wu Lien teh [15] Kalabuchov & Raevsky [91])

3 The *Meriones meridianus* of south east Russia are non hibernating animals which leave their burrows mainly at night They are ratlike in appearance but their hind legs are longer and stronger Their burrows are shallow and have several hidden entrances in addition to the principal one The latter is kept closed with sand by the female the sand being accumulated by several strokes of the hind legs The female discontinues blocking up the entrance when her young mature in order to let them go out on the surface The mating season of these fertile animals extends from May to October pregnancy lasts from 25 to 28 days Though up to three litters may be born to one female per year 85 % have only one litter annually The young leave the maternal burrows after 25 to 30 days (Rall [16])

As stated by Baltazard et al [4] the *Meriones* of the Iranian Kurdistan keep away from man and dig their burrows in non cultivated areas far from settlements They are of sedentary and peaceful habits frequently visiting the burrows of their neighbours particularly during the mating season Though accumulating food reserves for the winter they leave their burrows even during the coldest weather

4 The *Tatera brantsi* which together with the Namaqua gerbil *Desmodillus auricularis* form the main plague reservoir in South Africa are active non hibernating animals of strictly nocturnal habits Their size is approximately that of rats Their burrows have many entrances and are arranged in colonies the distribution and size of which vary according to the nature of the soil and the available food supply There are two main breeding seasons—one after midwinter towards spring and one after midsummer towards autumn Three to five young are born per litter they leave the parent burrows when maturing and may range over a wide area before settling down to breed at the age of three months (Wu Lien teh [15] Davis [39])

According to Powell (quoted by Schulz[173]) the Namaqua gerbils are less active and less sociable animals than the *Tatera* species They live in families of 2 to 8 members, inhabiting warrens which have many entrances They reach sexual maturity at the age of three months, and have four litters annually of 4 to 6 young per litter

As stated in chapter 1, the coucha rat, *Rattus natalensis* a fertile, mouselike animal of nocturnal habits, plays an ominous role as a link in the transition of plague from the gerbils to man Being rather inactive these rats prefer occupying burrows deserted by gerbils or other rodents to providing their own shelters Since they prefer to frequent, rather than shelter permanently in, human settlements and houses, and may even live away from human habitations these animals seem to deserve a place among the wild rodents (Davis [37] Fourie [53] [54])

5 The *ground squirrels of the western States of the* USA fall according to Eskey & Hass,[48] *into three size groups*

(i) large animals comparable in size to the grey tree squirrels e g *Citellus beecheyi*, *C columbianus*, and *C variegatus grammurus*,

(ii) medium sized animals somewhat more than half the size of the larger species, including *C armatus*, *C beldingi*, *C richardsoni* and *C washingtoni*,

(iii) small ground squirrels including among others *C spilosoma* *C townsendi*, and *C tridecemlineatus*

The prairie dogs (*Cynomys*) are comparable in size to the large ground *squirrels and may be divided*, according to Eskey & Haas,[48] into two groups—the white tails, comprising *Cynomys leucurus*, *C gunnisoni* and *C parvidens*, inhabiting the Rocky Mountains, and the black tails *C ludovicianus*, which range over the Great Plains The former live in a *manner similar* to that of the ground squirrels and often on common *ground* with them the latter live in peculiar circumscribed colonies or towns , *marked by prominent* mounds and the absence of all low vege *tation in the immediate vicinity*

Eskey & Haas state that the wood or pack rats (*Neotoma*) also fall into two groups bushy tailed species such as *Neotoma cinerea*, living in the *more forested* country of the colder zones and found infected in California, and round tailed species, found in the southern deserts, among which, according to these authors, *N desertorum* plays the most important role It should be noted that these rats like most rodent species found in the southern deserts, are of strictly nocturnal habits

The *Peromyscus*, suspected by Mohr [138] to be of etiological importance are white footed meadow mice, apparently apt to frequent the outbuildings of farms (Meyer & Holdenried [134]) The *Lagurus curtatus* recently incriminated as an important plague reservoir in Washington State by Link,[105] is a vole inhabiting sage brush areas

Dealing generally with the ecology of the rodent species found involved in the western States of the USA, Eskey & Haas [45] emphasized that

> human habitation and agriculture produce conditions attractive to many wild rodents. In irrigated valleys, around orchards, or in camps and resorts the various species often congregate, easily adapting themselves to an association with humans and enjoying the artificially enhanced food supply.

While such species are apt to shelter in haystacks, empty spaces beneath buildings, lofts, and other man-made structures, the rodents which stay away from human settlements generally live in tunnels, the location and architecture of which varies with the species. Most of these rodents congregate in colonies, though in a degree differing according to the species. However, the observations of Brown [21] in Alberta, Canada, prove that there are exceptions to this rule. Brown found that *C. richardsoni* did not live together but were widely scattered and moved extensively over the prairie, whereas *C. columbianus*, common in the mountains and foothills, lived in colonies and moved within a restricted range.

Generally speaking, the wild rodents in the western States of the USA appear to produce a single litter each year, the number of offspring varying from four to a dozen. The young remain in the nests for the first few weeks of their life, after which they build tunnels of their own and forage for themselves (Eskey & Haas [48]).

It is of great importance that many of the species undergo hibernation or aestivation or a combination of both. It appears, however, that such periods of inactivity do not occur as regularly and universally as in the case of the tarabagans and of the susliks in south-east Russia. Meyer [130] noted in this connexion that the young ground squirrels did not hibernate or aestivate, while Evans & Holdenried [49], working at the Calaveras dam in California, found that

> "there was evidence of both aestivation and hibernation for varying periods but not all of the squirrels were inactive at the same time."

Meyer [1 8] laid some stress on the cannibalistic tendencies of the rodents found plague-affected in the western States of the USA and pointed out that "involvements of the lymph nodes adjacent to the upper and lower gastrointestinal tube as a sequel of cannibalism are by no means infrequent." Though these findings deserve attention, one cannot conceive that this mode of infection could have played a really important role.

6. *Heteromys anomalus*, incriminated together with *Sigmodon hirsutus* as a primary plague reservoir in Venezuela, is stated to frequent cultivated fields, granaries, and even houses in search of food, whereas the latter rodent lives in forests, rarely visiting fields and never entering houses (Isaac Riaz [56]).

7. The "cuis" (Caviinae), which play a principal role in the wild rodent plague foci in Argentina, generally live underground but may make

tunnels between plants in locations where the vegetation is sufficiently abundant for this purpose (Barrera [7]) These animals do not penetrate into occupied buildings and, in general, seem to have little contact with man (Macchiavello [119]) However, exceptions to this rule seem to exist, Barrera,[7] for instance, noted that, in the north of the country the Indians used the "cuis" as food The breeding season of these species is usually in spring

Graomys griseoflavus (which probably also serves as a reservoir host in Argentina), although originally an arboreal rodent, may nest in various locations, even under the roofs of houses As stated by Barrera [9] when dealing with the manifestations of wild rodent plague in Mendoza Province, these rodents were hunted for their furs as well as for culinary purposes

8 Discussing the plague focus in Huancabamba Peru, Macchiavello [119] stated that the *Akodon* (Cricetinae) which, together with an *Oryzomys* species, formed the primary reservoir of the infection, were attracted to the farms at harvest times In the Peru Ecuador border regions humans contracted the infection in the fields where the secondarily involved Cricetinae lived *Sciurus stramineus nebouxi*, which formed the primary reservoir, was an arboreal rodent

Movement range

The movements of free living animals like the wild rodents fall into two categories

(1) a normal dispersal' (Meyer [1 9]) caused by physiological needs such as the daily requirements for food the search of the maturing young for shelter, and the periodical movements of groups to the vicinity of fields or even settlements at harvest times ,

(2) migrations due to abnormal causes e g , food scarcity, or catastrophes such as floods, which bereave the animals of their shelters as well as of their food supplies

It is impossible to make a clear cut distinction between these two categories as far as the range of the movements is concerned As already noted, the young, when in search of homes, and even the adults (especially the males) of some species, may roam quite far Still more important certain species which play a subsidiary role in wild rodent plague may normally cover wide distances This is true in particular for the Lagomorpha, the South African hares, for instance travel far in the course of a single night

That large scale true migrations of wild rodents do take place is well confirmed by the observations made of the lemmings in Scandinavia (Elton [47]) Evidence is also available that migrations undertaken on a minor scale have helped the spread of wild rodent plague For instance

Barrera[8] stated that the *Graomys* driven by hunger to human habitations were responsible for a plague outbreak in northern Argentina Similarly multimammate mice were considered to have played an important role in the 1944 5 plague epidemic in Ngamiland Bechuanaland Protectorate At the height of the gerbil epizootic the multimammate mouse population became unusually large a flood forced them to leave their burrows in the swamps and they invaded the villages bringing with them the infection contracted from the gerbils

It would appear, however that such instances of a spread of wild rodent plague through true migrations are exceptional

Susceptibility and resistance

As stated earlier laboratory tests have proved that different wild rodent species may vary markedly in their susceptibility to infection with *Pasteurella pestis* Several observers have claimed that the differences found to exist in this respect are not merely of academic interest but are of great actual importance Tikhomirova[199] denied that the highly susceptible sisels (susliks) could be the preliminary plague reservoirs in south east Russia and incriminated the rather resistant *Meriones meridianus* as the fons et origo mali Similarly Krumbiegel[9] stated that compared with the suslik *Citellus pygmaeus* *C fulvus* was of no great importance in the perpetuation of plague in south east Russia because it succumbed to a fulminant type of the infection which could therefore not persist long in this species or spread far On the other hand Davis[40] noted in South Africa that epizootics in the Namaqua gerbil *Desmodillus auricularis* (a species found by Pirie[153] to be rather resistant to plague) took a pro tracted course which no doubt favoured a perpetuation of the infection

In a recently published paper Baltazard et al[4] not only stated that the *Meriones* subspecies—which were somewhat resistant to infection with *P pestis*—were an important reservoir of the disease in the Iranian Kurdistan but also maintained that in general slightly susceptible rather than highly sensitive species were instrumental in the perpetuation of wild rodent plague

The occurrence and importance of seasonal variations in the suscepti bility to the infection within any one species have been claimed by observers in south-east Russia as well as in the western States of the USA

In this connexion mention may be made of the investigations made in south east Russia by Nikanoroff[144] to study the influence of the seasons on experimental plague in the sisels (susliks) Starting his experiments in the middle of June he successively tested four batches of susliks each consisting of 30 animals at fortnightly intervals by subcutaneous injection with uniformly virulent cultures While most of the animals in the first batch quickly succumbed to acute plague the disease displayed an increas ingly slow evolution in the three subsequent batches Thirty days after

infection there were no survivors in the first group as compared with 3 in the second, 5 in the third, and 18 in the fourth

In 1934, Tinker & Kalabuchov[70] found that young ground squirrels born in that year were most susceptible to plague adult females less so and adult males least The two workers assumed that these differences were related to deteriorations in the physiological condition of the animals found to evolve in the females during gestation, and in the young animals during the period of dispersal

Carrying out laboratory tests with *Meriones meridianus* Lobanov & Fedorov[108] noted that the gerbils infected during a period from April to July showed a localized and apparently resolving type of plague characterized by the presence of minute abscesses which became encapsulated and were eventually replaced by scar tissue, cultivation and animal experiments gave negative results in such instances The majority of the animals infected during a period from July to October showed an acute and generalized form of the disease

The presence of a most acute form of plague among the young ground squirrels in California was claimed by Harrison[0] and was confirmed by Meyer,[130] who stated that laboratory tests had proved that the young animals were highly susceptible to the infection He also maintained in contrast to Tinker & Kalabuchov,[07] that the adult males, and not the adult females, came second in order of experimental susceptibility

Although the evidence quoted above is noteworthy, it is the present writer s feeling that the higher susceptibility of young wild rodents might sometimes have been more apparent than real Their chances of infection are particularly great because they may roam far during the period of their dispersal, and also because they may settle down in burrows containing infected fleas the former inhabitants having succumbed to the disease The outcome of the laboratory tests might have been the result not of an increased susceptibility of the young animals, but of a decreased susceptibility of the adults due to aestivation or approaching hibernation This may have been particularly true in the case of the Californian squirrels, the young of which, in contrast to the adults neither aestivate nor hibernate

Role of hibernation

The importance of hibernation in the epizootiology of wild rodent plague has been proved by several observers

1 *Siberian marmots (tarabagans)* The idea that plague infection might remain quiescent in the bodies of hibernating tarabagans and that it might kill the animals after they awaken in the spring seems to have been first put forward by Le Dantec[103] Experimental support for this hypothesis was obtained by Dujardin Beaumetz & Mosny[4] who found in two alpine

marmots (*Marmota marmota*) which had been infected while hibernating and which died after 61 and 115 days respectively foci of chronic pneumonia teeming with plague bacilli

These results were fully confirmed by further experiments recorded by Wu Lien teh[1, 14] Wu Lien teh & Pollitzer[16] and Gaiski[58] which definitely proved that

(*a*) Siberian marmots infected with *P pestis* while hibernating may continue to sleep and not succumb to a generalized infection until after awakening in spring in the instances recorded by Wu Lien teh[2, 14] the longest periods of survival were 88 and 130 days respectively

(*b*) animals infected percutaneously or subcutaneously during hibernation and killed at various intervals during further sleep may show signs of resolving plague or evidence of a peculiar form of latent plague in which virulent bacilli persist at the site of inoculation and/or the regional lymph nodes

No doubt can exist that the persistence of such a localized infection in the hibernating animals was responsible for the appearance of generalized plague after they awakened in spring

Gaiski[58] assumed that the peculiar evolution of plague in the hibernating tarabagans was in causal connexion with the process of bacteriophagy However though he had been able to demonstrate the presence of plague phages in some of his experimental animals this assumption cannot be considered as generally valid The extensive work carried out by Wu Lien teh and Pollitzer with both hibernating and non hibernating tarabagans produced no evidence of the presence of plague phages

A most interesting point established by Gaiski[8] was that three tarabagans which had been infected while hibernating rapidly succumbed to an acute type of the disease when re infected with *P pestis* soon after they awakened In all three animals localized signs of the past infection were noted at autopsy As Gaiski stated these observations explained why in spring animals which had been infected during hibernation and had carried plague bacilli to the moment of awakening may succumb

It is curious to note in this connexion that according to the observations of Rudneff,[173] hibernating sisels (*C pygmaeus*) showed during hibernation a leucopenia with a specially marked diminution of the neutrophiles

2 *Susliks* Some observations on the evolution of plague in hibernating susliks seem to have been made by early investigators in south east Russia for an editorial appearing in the *Lancet*[99] in November 1913 stated that it has been shown by experiment that infection of this animal [suslik] may be greatly prolonged especially during the hibernating season "

Churilina[8] also reported that hibernating susliks when plague infected were apt to survive up to five months while the controls died in two to seven days

Further evidence was obtained by Gaiski[56] who studied during the course of a year the seasonal changes in the susceptibility of the susliks

to plague He used for this purpose 242 animals, grouped in 27 batches infecting each succeeding group with a plague strain isolated from the preceding one Seasonal differences were noted in two directions

(i) the mean length of illness varied showing a minimum (3 days) in June and a maximum (25 days) in winter

(ii) in June and July 100% of the animals had bacteraemia as compared with 60% in winter and 40% in March The other animals either had a localized form of plague with bacilli confined to the site of infection or harboured the causative organisms in their organs but not in their blood The incidence of the localized type of the disease was highest in winter (30%)

Of the 30 susliks infected during hibernation

21 awoke and succumbed after 2 to 22 days (the average was 8 days)
3 were killed after infection (2 after 15 days, and 1 after 35 days),
6 succumbed after 45 to 138 days

Of the three animals killed, two (one killed after 15 days the other after 35 days) showed latent plague with positive findings at the site of the infection only, the third animal showed bacteraemia

Three animals died after 96, 120, and 138 days, respectively, at the end of hibernation Two of these showed abscesses at the site of infection while in the third animal plague bacilli were present in the internal organs as well as at the site of inoculation

Some field observations made in south east Russia seem to lend support to the experimental evidence furnished by Gaiski [56] Thus, Tumansky [206] noted the existence of two periods when plague assumed epizootic proportions among the susliks—a major one commencing at the time of the dispersal of the young and lasting until the onset of hibernation, and a minor one in early spring at the time of mating soon after the end of the winter sleep

It is of interest to note in this connexion that, according to the inhabitants of Mongolia and Transbaikalia spring outbreaks of plague did occur among the tarabagans However, these statements ought to be received with caution because instances of a high mortality in spring due to other causes have been observed in these animals [15]

3 *North American ground squirrels* Two series of experiments undertaken by Prince & Wayson [159] gave the following results

(*a*) two hibernating ground squirrels (*C richardsoni*) were inoculated with plague One succumbed to an acute form of the infection after two weeks The second—and two further animals which had been infested with plague infected fleas—did not contract the disease

(*b*) out of four ground squirrels (*C townsendi*) which survived four months in hibernation after intracutaneous infection with *P pestis* one sickened seven days after awakening and died on the eighth day showing an acute inflammatory reaction at the site of inoculation and bacteraemia The plague nature of the process was

fully confirmed. The three other animals remained well and were killed 15 days after awakening. They appeared normal at autopsy.

A point of great interest is the relation between the latent type of plague characterized above and the "inapparent" form of the infection referred to in chapter 4. As stated, it is so far not justifiable to identify these two types. Up to the present it has merely been claimed that inapparent plague may lead to a generalized infection while in the case of latent plague positive proof of such an evolution has been obtained.

Some evidence is available which shows that, as has been fully proved in the case of the commensal rats, in wild rodents also the prolonged existence of plague may lead to a progressive increase in the number of animals which are resistant to the infection.

Noting that in plague-stricken localities of California the ground squirrels were rather resistant to laboratory infection with *P. pestis* while those captured in plague-free districts were uniformly susceptible, McCoy[126] expressed the view that the preponderance of insusceptible animals in the foci

"may mean a gradual extinction of the disease or it may indicate that this partially resistant race of rodents will, if not vigorously attacked, perpetuate the disease for many years."

The early findings of McCoy were fully confirmed by Meyer[130] by the examination of approximately 450 healthy ground squirrels collected partly from a known plague focus and partly from an area where the disease had not been demonstrated. Meyer did not feel sure that the insusceptibility to infection with *P. pestis* found in the rodent populations of plague foci was solely the result of a process of "natural selection" by which the receptive strains of the animals were gradually wiped out while the resistant strains survived. He suggested that an immunity induced in individual animals by previous plague attacks might also be of some importance. It also seemed significant that, according to the observations of Bychkov [illegible], guinea pigs which had not become manifestly ill when bitten by plague-infected fleas had proved resistant to subsequent challenge infection with *P. pestis*.

Evaluating the actual importance of the insusceptibility becoming increasingly manifest in the rodent population of plague foci, Meyer[130] aptly summed up the situation as follows:

"There is every reason to believe that the interplay between infection and immunity may be influenced by an infinite variety of ecological factors which accelerate or retard it. For example, even a coalescing epidemic spread of the plague infection in a rodent population may fail to reach every colony. Thus, at the apparent termination of the epizootics, both susceptible and resistant rats or squirrels may survive and interbreed, furnishing sufficient hosts to maintain the infection in a smoldering non-readily recognizable state."

Seasonal incidence of plague

That wild rodent plague often shows a seasonal incidence being active during certain months only and more or less quiescent during the rest of the year has been proved by many observations some of which have shown that in addition to the rodents themselves, their fleas may play an important role in this respect

As far as the part taken by the rodents is concerned, stress has already been laid upon seasonal changes in the susceptibility to infection with *P pestis* found to exist in some species Mention has also been made of two extrinsic factors which are apt temporarily to exacerbate plague incidence—namely

(1) the overcrowding of the burrows in late spring and early summer due to the presence of the young as well as the adult animals

(2) the dispersal of the young which for the reasons previously enumerated greatly enhances their chances of infection

It is clear that in hibernating species plague can remain manifest during the warm season only However as discussed earlier the infection is apt to persist in the hibernating animals in a latent form and also as will be discussed in a future chapter in the rodent fleas

Since in the western States of the USA only the adult squirrels hibernate and/or aestivate (the young animals and the non hibernating species involved in plague outbreaks remain active throughout the year) the seasonal incidence of plague in these areas was less clear-cut than in the regions where the tarabagans or susliks were the reservoir hosts Indeed Meyer[130] stated that plague was sometimes found to be active among ground squirrels in December and January Generally speaking however summer epizootics prevailed and the lesions observed in squirrels shot in winter are those of subacute and resolving plague

In the wild rodent foci of Argentina epizootics prevailed in winter but as pointed out by Barrera[10] *this seasonal incidence was mainly due* to a low flea index prevailing in summer However a decrease in the rodent population, caused by epizootics during the preceding winters was also apt to reduce the incidence of the disease during the warm seasons

In regard to South Africa it was stated by Davis[33] that

"man is at risk of infection during the summer plague season whereas rodent epizootics whether in wild or domestic species do not show any marked seasonal incidence"

Trend of epizootics

Reporting on the 1935 anti plague campaign in the Bechuanaland Protectorate Gerber[61] stated that it usually took 8 to 12 months for an epizootic to burn itself out About 90% of the wild rodents (mainly gerbils)

Effect of density of wild rodent populations

It is obvious that

(*a*) if plague spreads to or reappears in a locality populated by wild rodents the situation is far more likely to become serious if the rodent population level is high than if the rodents are scarce

(*b*) in the case of wild rodents living in colonies, an intercolonial spread of the infection will be facilitated if these colonies are close together and *impeded if the colonies are widely separated* ,

(*c*) severe epizootics in a highly populated area may reduce the number of wild rodents to a level comparable with that of sparsely populated districts and thus curb the further progress of the infection

It is important to note in this connexion that the density of the rodent population in any given group of burrows and colonies is not uniformly high throughout the year, but reaches a maximum during the period between the birth and dispersal of the young Thus according to Kalabuchov & Raevski,[9] the population level in the sisel colonies of south east Russia was 3 2 to 3 6 times higher during this period than during the rest of the year These authors maintained that, if plague was pre existent or was introduced at that time, the temporary overcrowding of the burrows considerably aggravated the situation and was at least partly responsible for the high mortality (85%) of the susliks during their first year of life

As has been established by investigation of the epizootics or has been suggested by studies on the occurrence of human outbreaks the incidence of plague in the wild rodent foci often showed periodic fluctuations the peaks of which generally coincided with a maximal abundance of the rodents concerned The length of these cycles was usually 3 to 5 years, but was sometimes more extended (Wu Lien teh , [13 215] Fourie ,[54] Meyer ,[130] Davis ,[39] Macchiavello [119])

These observations attracted much attention since extensive studies on the population fluctuations of wild mammals particularly of the lemmings, had proved that periodical epizootics were of paramount importance in regulating the numbers of these animals As summarized by Elton [47]

> the method by which most rodents regulate their numbers is as follows increase in numbers over several years up to a point at which an epidemic of some sort occurs which kills off a large proportion of the population Increase then takes place again *and is followed by another epidemic and so on indefinitely*

The question naturally arose as to whether plague was one of the epidemic or, one should rather say, epizootic diseases apt to act as a population regulator While this was considered likely by some workers e g, Wu Lien teh, [213 215] Barrera,[8] Meyer,[131] and Evans & Holdenried,[49] Davis[38] definitely spoke of the establishment of so potent a regulatory factor as plague in the South African wild rodent foci

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That wild rodent plague often shows a seasonal incidence being active during certain months only, and more or less quiescent during the rest of the year has been proved by many observations some of which have shown that in addition to the rodents themselves their fleas may play an important role in this respect

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Reporting on the 1935 anti plague campaign in the Bechuanaland Protectorate Gerber[61] stated that it usually took 8 to 12 months for an epizootic to burn itself out About 90% of the wild rodents (mainly gerbils)

were killed, and about three years elapsed before the remainder increased to the previous density Epizootics then reappeared

An excellent description of the "epizootic cycle in *Tatera brantsi* foci was given by Davis[39] thus

> The development of epizootic conditions is preceded by sporadic outbreaks of epizootic plague in a few smouldering foci The gradual diffusion of *P pestis* from these initial foci involves more and more colonies until the majority are in various stages of decline It takes from 4 to 6 months for an isolated colony to die out but as cross-infection from one colony to another is erratic it may take 12 or 15 months for a major epizootic to run its course Major epizootics recur on the average every 5 to 6 years Man is at risk of infection before during and after this major epizootic which in effect, means for at least 3 in every 6 years

A more detailed description of the evolution, course, and decline of the epizootics in the South African gerbils has recently been given by Schulz[1 5] who stated that, after a major epizootic, the gerbil population was restored to normal density in 3 to 5 years

Investigating ground squirrel epizootics in Kern County, California, Evans et al[50] found that these exacerbations of the plague situation ' coincided fairly well with the period of dispersal of the young squirrels The epizootics ran a separate course in each of the localities involved, the active phase apparently lasting for 2 to 3 weeks only

Persistence of infection

It may be gathered from the preceding discussion that the persistence of wild rodent plague depends upon the establishment of a kind of equilibrium between the various factors favouring or counteracting the establishment of the causative organism in the host herds

No doubt can exist that in some of the species involved a reduced *susceptibility of the animals to infection with P pestis* is instrumental in creating such an equilibrium This mechanism is at work in the case of species such as the Namaqua gerbils of South Africa the *Meriones meridianus* of south east Russia, and also, as recently claimed by Baltazard et al,[4] in case of the *Meriones* forming the plague reservoir in Iranian Kurdistan

While fully admitting the great importance of this mechanism for the perpetuation of plague in some of the wild rodent species involved the present writer cannot agree with the thesis of Baltazard and his colleagues that the presence of such a reduced susceptibility of the host herds is a sine qua non for the continuation of the infection For, as shown below, a persistence of the infection even in fully susceptible species, is rendered possible in various ways

A most important stabilizing influence is exerted in some of the fully susceptible wild rodent herds by the hibernation period which, while cutting short the decimation of the species through acute or subacute plague

at the same time permits a carry over of the infection in the form of latent plague

Although this regulating mechanism is absent in the case of non hibernating species several other factors exert a similar influence As has been noted even in the non hibernating species plague often shows a seasonal incidence i e , periods during which epizootics are apt to occur alternate with seasons during which the infection, assuming an enzootic character, becomes far less fatal

The decrease in the rodent populations during severe epizootics and the erratic progress of the latter which often spares individual colonies within the affected areas form important means of preventing a whole sale extinction of the hosts

On the other hand during the off seasons when the continued existence of the invaders is threatened there will usually be a sufficient number of rodents which because suffering from acute or subacute plague with bacteraemia are capable of serving as links in the perpetuation of the infection Moreover even if this mechanism should fail fleas which con tinue to harbour *P pestis* are apt to carry over the infection

Wild rodent species other than the primary reservoir hosts may also take part in the perpetuation of the infection Their involvement is bound to become more serious at the same rate as the intensity of plague increases among the primarily affected species Consequently if the numbers of the latter are reduced to such a degree as to lessen the perpetuation of the disease the subsidiary species are apt to be sufficiently involved to carry over the infection until the primary hosts become numerous once more

As is suggested by an interesting observation of Meyer[13] species which formerly played a subsidiary role in a focus of wild rodent plague may under exceptional circumstances become the primary reservoirs of the infection He recorded in this connexion that in a focus originally maintained by *Citellus beecheyi*

> under the influence of a control program which was directed solely against the squirrel *Pasteurella pestis* had transferred its activities to the mice (mainly *M[illegible] californicus*) and thus had protected its persistence and perpetuation "

Discussing the perpetuation of wild rodent plague in south-east Russia Nikanoroff[146] and Gaiski[57] laid stress upon the existence of a " transi tional zone between the northern steppes where sisels (susliks) were the primary hosts and the southern sandy stretches where in their opinion mice were principally involved They maintained that in this border region summer epizootics among the susliks were regularly [illegible] by winter outbreaks among the mice Thus a vicious circle [illegible] the perpetuation of the infection seemed to exist

Tikhomirova[18] while confirming that the epizootics in *M[illegible]* *[illegible]* which she considered as the reservoir host in the [illegible]

regions, took place in autumn and early winter did not share th in the existence of a transitional zone, but maintained that a sp plague northwards from the *Meriones* foci led to secondary infec the susliks

In a note entitled 'Persistence of sylvatic plague', Meyer & E recorded an observation in San Mateo County, California accor which

> two of the locations in the County where infected [ground squirrel] fleas we in 1936 were recognized as the same colony or series of burrows proven to harbor squirrels in the summer of 1916

They added that "these and similar observations indicate that s plague persists probably indefinitely in an area once invaded "

Though this is undoubtedly the case, it does not seem very like the same burrows or colonies remain continually infected thro prolonged periods

Spread of infection

Though as previously discussed a spread of wild rodent pla distance may be effected through the agency of migrations or of a like hares which normally travel far, these and similar means of disse ing the infection per saltum are of comparatively little importance, or b operative only under exceptional circumstances as holds true migrations

Ordinarily, the spread of wild rodent plague is by contiguity probably to an accumulation of small movements among rod (Meyer [130])

Discussing this problem Davis [39] drew an apt distinction betwe intracolonial and an intercolonial spread of the infection, stating th

> the movement of individuals brings each warren a contact by relay movement a night's activity In consequence, the spread of plague throughout the warren colony is not hindered but the spread from one colony to another mainly by males and by the maturing young at rather rare intervals is sporadic

That wild rodents may be carried to settlements by rail is suggest an observation in the port of Quequen Argentina,[19] where cuis (*leucoblephara*) were found in the grain supplies imported from the h land It was assumed that the transportation of grain by rail was re sible for the rat epizootic present in the port at the time

As stated in chapter I, the epizootic prevailing among the comm rats of Tacoma Washington in 1942 3 was suspected to have been d an importation of plague from wild rodent foci in the hinterland bu noteworthy that Hundley & Nasi [8] spoke in this connexion of the fin of rats and mice in the grain cars That however possibilities of a trans

ation of plague infected wild rodents do exist in the western parts of the USA is shown by recent observations of Ecke & Johnson[43] in Colorado. As pointed out by these workers, ranchers in the southern part of the State prompted by the desire to control the prairie dogs on their property were prone to pay attention to epizootics among these rodents in New Mexico and to fetch diseased animals from there in order to release them on their land. Ecke & Johnson obtained definite knowledge of three instances of this kind, in one of which the rancher in question drove 250 miles from his home to get diseased prairie dogs. According to them two workers

"reliable sources state that control by the above method was very good as may be expected. It is evident, however, that the ranchers are unaware of the dangers to which they are exposing themselves by practicing this form of prairie dog control. If this practice is as common as is believed, it probably is the explanation for some of the abnormal jumps of plague epizootics over great distances."

Ecke & Johnson also referred to a rancher's boy reported to be catching prairie dogs from an epizootic zone and transporting them to a nearby town to be sold as souvenirs to tourists" but considered this practice as very uncommon.

In South Africa great stress was laid on preventing the transportation of infected rodents by rail or even trucks (Thornton[19]) but one must wonder whether the transportation of wild rodents was frequent. According to Fourie[53] coucha rats (*R. natalensis*) were seldom carried in farm produce. That field rodents may be transported in this manner is proved by an observation in the Cumbum Valley of India where four such animals were detected among rice bags on bullock-carts[83] but this was apparently an exceptional occurrence.

In a study on "The focality of rodent plague in the light of ecologo-geographical ideas" Rall[163] distinguished between "mechanical" barriers against the spread of infection, such as rivers and mountain ranges, and "biological" barriers created through the presence of "sterile zones" where susceptible rodent hosts, if present at all, occurred either in small numbers or were patchily distributed.

The importance of such biological barriers is illustrated by the observations made by Fedorov et al[51] of a sandy stretch on the left bank of the Ural River. Plague did not become established in this locality because the latter was sparsely populated by susliks and mice and also, as Fedorov and his co-workers believed, because the seasons during which the various rodent species became comparatively numerous did not coincide.

However, observations in South Africa particularly[illegible] have shown that zones which were ecologically unsuitable for the establishment of plague or belts which had been artificially freed from rodents did not absolutely bar the spread of the infection because they may be overrun or outflanked by waves of severe epizootics.

regions, took place in autumn and early winter, did not share this belief in the existence of a transitional zone but maintained that a spread of plague northwards from the *Meriones* foci led to secondary infection of the susliks

In a note entitled "Persistence of sylvatic plague , Meyer & Eddie[133] recorded an observation in San Mateo County, California, according to which

> two of the locations in the County where infected [ground squirrel] fleas were found in 1936 were recognized as the same colony or series of burrows proven to harbor diseased squirrels in the summer of 1916

They added that 'these and similar observations indicate that sylvatic plague persists probably indefinitely in an area once invaded

Though this is undoubtedly the case, it does not seem very likely that the same burrows or colonies remain continually infected throughout prolonged periods

Spread of infection

Though as previously discussed, a spread of wild rodent plague at distance may be effected through the agency of migrations or of animals like hares which normally travel far, these and similar means of disseminating the infection per saltum are of comparatively little importance, or become operative only under exceptional circumstances as holds true of the migrations

Ordinarily, the spread of wild rodent plague is by contiguity, due probably to "an accumulation of small movements among rodents (Meyer[130])

Discussing this problem, Davis[39] drew an apt distinction between an intracolonial and an intercolonial spread of the infection stating that

> the movement of individuals brings each warren a contact by relay movement during a night s activity In consequence the spread of plague throughout the warrens of a colony is not hindered but the spread from one colony to another mainly by adult males and by the maturing young at rather rare intervals is sporadic"

That wild rodents may be carried to settlements by rail is suggested by an observation in the port of Quequen, Argentina[19] where cuis (*Galea leucoblephara*) were found in the grain supplies imported from the hinterland It was assumed that the transportation of grain by rail was responsible for the rat epizootic present in the port at the time

As stated in chapter 1 the epizootic prevailing among the commensal rats of Tacoma Washington, in 1942 3 was suspected to have been due to an importation of plague from wild rodent foci in the hinterland but it is noteworthy that Hundley & Nasi[8] spoke in this connexion of the findings of rats and mice in the grain cars That, however possibilities of a transport

ation of plague infected wild rodents do exist in the western parts of the USA is shown by recent observations of Ecke & Johnson[43] in Colorado As pointed out by these workers ranchers in the southern part of the State prompted by the desire to control the prairie dogs on their property were prone to pay attention to epizootics among these rodents in New Mexico and to fetch diseased animals from there in order to release them on their land Ecke & Johnson obtained definite knowledge of three instances of this kind in one of which the rancher in question drove 250 miles from his home to get diseased prairie dogs According to them two workers

"reliable sources state that control by the above method was very good as may be expected It is evident however that the ranchers are unaware of the dangers to which they are exposing themselves by practicing this form of prairie dog control If this practice is as common as is believed it probably is the explanation for some of the abnormal jumps of plague epizootics over great distances

Ecke & Johnson also referred to a rancher s boy reported to be catching prairie dogs from an epizootic zone and transporting them to a nearby town to be sold as souvenirs to tourists but considered this practice as very uncommon

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reach a maximum of 52% in 1947, and then gradually declined It will also be noted that, in 1948, 42 of these rodents were found infected as against 2 *Rattus rattus rufescens* However, in 1949, only a few specimens of each species proved positive and no instances of rodent plague were found in 1950 and 1951

TABLE XVI PERCENTAGE INCIDENCE OF RAT SPECIES IN BOMBAY MUNICIPAL AREA DURING THE PERIOD 1938 51

Year	*R rattus rufescens*	*R norvegicus*	*Gunomys kok*	*Bandicota malabarica*	Incidence of rodent plague
1938	24.7	25.4	27.3	1.2	—
1939	23.8	24.4	35.5	1.2	—
1940	24.9	23.9	34.8	1.0	—
1941	24.2	23.0	37.1	0.8	—
1942	20.9	22.5	45.7	0.8	—
1943	20.3	22.7	46.3	1.1	—
1944	21.9	22.1	47.0	1.1	—
1945	21.0	21.6	49.2	1.8	—
1946	19.6	23.7	49.0	1.1	—
1947	18.9	23.0	52.0	0.6	—
1948	23.7	18.3	47.4	0.8	2 *R r rufescens* and 42 *Gunomys kok* found infected
1949	23.3	20.0	42.2	0.4	4 *R r rufescens* and 2 *Gunomys kok* found infected
1950	23.9	17.6	39.3	0.7	—
1951	21.1	16.5	36.7	0.8	—

* *The figures for the period 1938 48 are taken from the reports of the Haffkine Institute Bombay while those for 1949 51 were kindly supplied by Dr P M Wagle Director of the Institute The rodents were reported to have been trapped alive only*

Some further observations on bandicoots were made in South India during the course of plague inquiries instituted by the Indian Research Fund Association (now the Indian Council of Medical Research)

Dealing with the results of this work in the Cumbum Valley, Madras Presidency (now Madras State), George & Webster [60] noted that bandicoots were occasionally found dead from plague in the villages and actually proved the presence of the infection in two *Gunomys kok* found near settlements Another report on this work even stated that

> it is usual in several villages of the Cumbum Valley for the epizootics to commence in bandicoots The infection then passes to house rats and mice [63]

According to Wu Lien teh [15] a statement to the same effect was made at the 1934 Conference of Medical Research Workers in Calcutta

George & Timothy,[59] reporting on a preliminary study of plague at a hill station in the Nilgiris also viewed the mole rats (*Gunomys kok*) and *Bandicota malabarica* with suspicion They stated that in that locality the mole rats lived around human habitations and sought shelter in the houses during rainy seasons or when food was scarce outside *Bandicota malabarica* on account of its semi domestic habits, seemed apt to act as the intermediary in the extension of plague from house rats to wild rodents However, though conditions seemed suitable for the establish ment of a sylvatic plague focus the two workers failed to find infected wild rodents

Later work at Coonoor in the Nilgiris led to the suspicion that, besides *R rattus* and some mouse species bandicoots (*Bandicota malabarica*) and mole rats (*Gunomys kok*) were also concerned with the spread of plague It was stated that the latter animals which were found in large numbers in the potato fields and compounds of dwelling houses, migrated into the houses when the potato season was over Their fleas were mostly *Stivalius* but occasionally other fleas including *Xenopsylla cheopis* and *X brasiliensis* were also found on them [8]

While no convincing evidence has so far been brought forward that the bandicoots of India play a role in the establishment and maintenance of sylvatic plague foci the findings made in Bombay City are of importance since they prove that plague susceptible rodents (other than the commensal rats and mice) which are able to accommodate themselves to a life near man may become dangerous not only because they are apt to act as inter mediaries in the spread of the infection but also because they may take the place of less susceptible rat strains in the houses

Mongolia north Manchuria and Transbaikalia As noted in chapter 1 plague evidently spreading from a wild rodent focus in Inner Mongolia became entrenched among the commensal rats of south Manchuria A similar evolution was presumably responsible for the frequent appearance of bubonic epidemics in Shan si and Shen si On the contrary in north Manchuria Outer Mongolia and Transbaikalia such a transition of the infection from the wild to the commensal species seems never to have been observed However Wu Lien teh [15] assumed that the absence of rat plague in Outer Mongolia and Transbaikalia was due to a scarcity of these animals Conditions for their involvement would have been more suitable in north Manchuria but the occasional appearance of the disease there was due to an importation through human agency resulting in a spread of pneumonic plague directly from man to man without involvement of the rats

South east Russia In south east Russia also the commensal rats did not seem to have become involved in the wild rodent epizootics [15] How- ever in the southern part of this area an important role was played by commensal mice *Mus musculus musculus* and *Mus musculus wagneri*

The latter, living mainly in the open during summer but retiring at the approach of cold weather, to haystacks and human dwellings (Tikhomirova & Zagorskaya [201]) played an important role in bringing the infection to man, particularly as they were in the habit of visiting the burrows of wild rodents (Obolenski [148])

Central Africa The interesting relationship existing in Central Africa between *R coucha ugandae* auctt (*R natalensis*) and *R rattus* seems to merit attention

As stated in chapter 1, *R rattus* formed the sole reservoir of the infection in Kenya and Tanganyika, as well as in those parts of Uganda where these rodents having arrived at a comparatively recent date, were able to replace the native *R coucha ugandae* It was stated [101] that *R rattus* not only sheltered in the thatch of roofs in the walls, and under the floors of the houses, but also sometimes lived, during the dry seasons, in sweet potato fields near the settlements The common field rat *Arvicanthis abyssinicus*, was sometimes seen to seek shelter in houses from which *R rattus* was absent and was then apt to harbour *X cheopis* in addition to its usual flea *X brasiliensis*

R coucha ugandae—the rodent by far the most frequently found in human habitations—was the plague reservoir in the focus at Lake Albert, *Belgian Congo, but, in the Lake Edward focus* seems to have shared this causative role with *R rattus alexandrinus* *Arvicanthis abyssinicus* was also regularly trapped indoors but although more conspicuous in the huts *of the Lake Edward focus, appears in that area, to have remained* free from plague so far

South Africa Both *R natalensis* and *R rattus* act as intermediaries between the primary gerbil reservoir and man The former—a rather inactive animal which prefers settling down in a deserted gerbil burrow to digging a new one and, at the same time has a strong tendency to enter human habitations or even to seek shelter in them—is particularly apt to act as an intermediary

The problem of the comparative importance of these two species is curious In his 1948 study on sylvatic plague in South Africa [38] Davis stated that

> the semi domestic multimammate mouse (*Mastomys coucha*) [scilicet *R natalensis*] is the intermediary between the primary gerbil reservoir and man The house rat (*Rattus rattus*) brings infection into even closer contact with man than the multimammate mouse The risk of plague to man is greatest in areas where one or both of these species com*monly frequent farm buildings* especially when they are abundant and in close contact with wild rodent colonies during an epizootic

Referring to this subject again in 1950, Davis [40] while once more emphasizing the importance of *R natalensis* as a link between the wild rodent reservoir and the commensal rodents and man stated that

"in South Africa *R rattus* stands at the end of the chain of infection from the sylvatic reservoir and is now largely responsible for human infections especially in the hyper enzootic areas"

At the same time Davis stressed that in South Africa, *R rattus*

does not however act as a *permanent* reservoir of plague in rural areas nor in urban areas where plague has from time to time shown itself It appears therefore that the climatic conditions are against the permanent circulation of *P pestis* in *R rattus* populations in spite of the fact that they are heavily parasitised with well known vectors

Referring to the plague manifestations in the Cape midlands during the period 1925 31 Davis[38] pointed out that both *R rattus* and *R natalensis* were absent from the central and western Karroo *Mus musculus* being the only commensal rodent present Consequently conditions for a spread of the infection to man were not as suitable as in the Orange Free State Human infection was mostly spread by means of a direct contact with wild rodents or their fleas

USA western States In their fundamental study on plague in the western areas of the USA Eskey & Haas[48] stated that the commensal rat species which were not indigenous to the western hemisphere, had been introduced into the Pacific regions more than 100 years ago As a result the rats became well established from the coast to the western ramparts of the Sierra Nevada Cascades enjoying both rural and urban distribution but they added most of the vast region lying between the Sierra Nevada Cascades and the Rocky Mountains –the great inter mountain plateau– is entirely rat free

On the eastern side of the Rocky Mountains commensal rats were numerous in all States except Montana In New Mexico the valley of the Rio Grande was well populated by these rodents as was the country east of this valley

It would seem that thus far no instance of rat plague has been recorded in the interior of the western States of the USA That this is not solely due to an absence of these animals is suggested by a recent observation of Link[104] Investigating an epizootic among the cotton tail rabbits of Lea County New Mexico he found commensal rats to be present but free from plague none of the 32 specimens trapped had fleas

In the coastal areas of the western States of the USA a spread of plague not only from the commensal rats to wild rodents but also from the latter back to the domestic species seems to have been observed As noted in chapter 1 it is usually assumed that an importation of plague into California by the sea route led first to rat plague the infection then spreading to the wild rodents However attention was drawn to the belief of some workers that pre existing sylvatic plague had secondarily involved the San Francisco rats It was also stated that a transition of the infection from the wild to the commensal species had probably been responsible for the 1924 Los Angeles epidemic and for the rat epizootics present

in 1942-3 at Tacoma, Wash During the former outbreak in which mainly *R norvegicus* were involved, a few infected ground squirrels were found but it was assumed that these animals might have become secondarily infected from the rats[215]

Attention was also drawn in chapter I to the fact that, on several occasions wild rodent fleas had been detected on rats and that, in one of these instances, Meyer & Holdenried,[134] working in California, succeeded in establishing the presence of plague in *R rattus rattus* and *R norvegicus* some of which were infested with ground squirrel fleas These authors stressed the potential danger of a spread of the infection from wild rodents living away from human habitations to species like *Peromyscus* and *Microtus* or to the commensal rodents all of which had, to some extent, close contact with man It should be noted however, that so far, instances of a transition of plague to the commensal rodents have been fairly infrequent, and that much attention is being paid to rodent and flea control in and around human settlements

Venezuela According to the descriptions given by Isaac Riaz[86] conditions suitable for an exchange of the infection between the commensal and wild rodents existed in the Aragua State of Venezuela Among the rats—which appear to have been immediately responsible for the infection of man—*R rattus rattus* in particular was often of peri domestic rather than of domestic habits, while *Heteromys anomalus*, one of the two wild rodents involved, frequented the fields and granaries and even entered the houses in search of corn Guinea pigs kept in houses (acures domesticos) seem to have been involved in the 1943 outbreak

Argentina As stated in chapter 1, the relations between wild rodent and rat plague were not uniform in the various plague affected areas of Argentina Since contact between the two groups of rodents was brought about only by the attraction of the wild rodents to the food supplies in settlements and houses, secondary involvement of the rats was bound to take place mainly in well cultivated areas In sparsely populated and little cultivated regions, e g, Mendoza Province, there was hardly any chance for a transition of plague from the wild rodents to the rats [10 1.]

Barrera[11] pointed out with much reason that the greatest potential danger of the transition of plague to the commensal rats was the possibility of an establishment of the infection in these animals which would lead to successive epizootics He claimed that such a stage had not been reached the major rat epizootics observed so far in Argentina having evolved in the coastal areas where no wild rodent plague existed

Quite naturally, human plague showed a sporadic incidence in the non cultivated areas where man could contract the infection only when entering the haunts of the wild rodents A secondary involvement of the

rats through contact with wild rodents invading the settlements led on the contrary, to the infection of groups of people[11 109]

Peru In chapter 1 attention was drawn to a curious observation made by Ramos Diaz[161] during a plague outbreak at Lambayeque The rats (*R rattus*) in this mountainous locality lived in the fields but visited the houses at night and thus came in contact with guinea pigs kept by the people No doubt the infection of the latter animals which was the immediate cause of the epidemic, had been derived from the free living rats

In the wild rodent plague focus in the Peruvian Ecuador border region commensal rats were altogether absent (Macchiavello[118 119]) The human victims contracted plague when harvesting corn in fields infested with Cricetidae which thus served as a link for passing the infection originally present in tree squirrels (*Sciurus stramineus nebouxi*) to man

Discussing the recently discovered plague focus in Huancabamba Peru Macchiavello[119] stated that there also commensal rats and their fleas were absent An invasion of the houses by the Cricetidae (the primary reservoirs of the infection) after the harvests was responsible for violent outbreaks of human plague

Ecuador As mentioned when dealing with the plague situation in Ecuador in chapter 1 in that country also the guinea pigs (*Cavia aperea*) kept in the houses, though often the immediate cause of epidemics, were merely instrumental in passing the infection perpetuated among free living commensal rats to man While Sáenz Vera[174] stated that the rats came in direct contact with the guinea pigs and even cohabited with them Macchiavello[117] came to the interesting conclusion that infection of the latter was effected by an invasion of the houses by the rat fleas rather than by the rats themselves

In 1936, when dealing with the problem now under review Wu Lien teh[215] maintained that generally speaking the danger of a spread of plague from wild to domestic rodents is more apparent than real

Bold as this statement seems no cogent reasons exist to refute it particularly if it is kept in mind that increasing emphasis is now being laid on reducing and even abolishing this danger by adequately controlling the commensal rodents

Commensal Rodents

Biology and ecology

Before entering into a discussion of the subject now under review an explanation is due as to why the term commensal rodents has been used in these studies in preference to the usual designation of "domestic rodents The reasons for adopting the former name—which has also been used by some other recent writers e g Schwartz[1 6]—are (*a*) to indicate that the common rats and mice though often forced in their struggle for

existence to shelter in human habitations, have really *nothing in common* with truly domestic animals, and (*b*) to indicate that it is by no means unusual for these rodents to lead a more independent existence instead of living in constant contact with man Since, even when away from man, they usually depend on food supplies destined for human consumption, the designation 'commensal' seems to remain appropriate

While, as discussed earlier (see p 253), several families or subfamilies and numerous species are involved in wild rodent plague apart from some local exceptions, the commensal rodents implicated in plague outbreaks belong to three species of Murinae—*Rattus norvegicus*, *Rattus rattus*, and *Mus musculus* As aptly maintained by Hinton [3]

> possibly these species are the most highly organized members of their family but unquestionably they are the most successful of mammals They are clearly of Asiatic origin but uninvited and unfortunately for us they have linked their fortunes with those of humanity Human enterprise, in all its phases and human negligence have disturbed the balance of Nature in favour of these species have afforded them an unnatural degree of protection from their many enemies a large and unmerited share of the world s food stuffs together with perfect travelling facilities Small wonder then that these creatures have invaded and colonized all lands , that they have developed into serious pests *taking a heavy toll from human prosperity*, and forming a most deadly menace to public health

Description

According to Hinton,[3] these three species of commensal rodents may be described thus

> *R norvegicus* is a large species of heavy and rather clumsy build , muzzle blunt ears small densely clad with fine and short hairs thick and opaque tail stout never as long as the combined length of the head and body (fig 25)
>
> *R rattus* is a smaller and slenderer animal of elegant build muzzle sharp ears large almost naked and translucent tail slender, at least as long as and often considerably longer than the combined length of the head and body (fig 24)
>
> *Mus musculus* is a small animal looking like a miniature *R rattus* ears moderately large clothed almost everywhere with short fine hairs tail about as long as the combined length of the head and body frequently longer rarely shorter

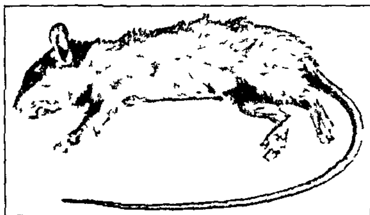
Other easily ascertainable characteristics of the three species are enumerated in table XVII, the measurements quoted referring to adult specimens [67, 209, 210]

As shown by table XVII, the coloration of the two rat species shows marked variations

Black varieties of *R norvegicus* are to be found, thus stultifying the name of brown rat which is often given to this species

The differences in coloration found in the case of *R rattus* have been used to distinguish between three subspecies—namely, *R rattus rattus* which is black or slate coloured on both back and belly , *R rattus alexandrinus*, which has a tawny back and a greyish white belly , and *R rattus*

FIG 24 RATTUS RATTUS RATTUS



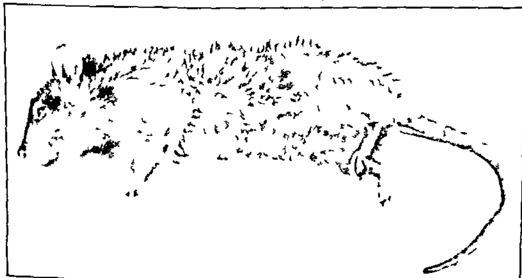
Si e 35 6 46 cm (including tail)

FIG 25 RATTUS RATTUS NORVEGICUS



Si e 33 47 cm (including tail)

FIG 26 BANDICOTA BENGALENSIS KOK (GUNOMYS KOK AUCTT)



Si e 39 8-47 cm (including ta l)

TABLE XVII CHARACTERISTICS OF ADULT COMMENSAL RODENTS

	R norvegicus	*R rattus*	*M musculus*
Body weight	10 17 ounces (280 5-484 5 *g*)	4 12 ounces (114 342 g) Usually not over 8 ounces (228 g)	Less than 1 ounce (28 5 *g*)
Length of head and body	7 10 inches (180 255 mm)	6½ 8 inches (165 205 mm)	2½-3½ inches (65 90 mm)
Colour	Grey brown but may be black	Black grey brown or tawny may have white belly	Brown grey
Length of tail	6 8½ inches (150 215 mm)	7½ 10 inches (190 255 mm)	3-4 inches (75 100 mm)
Hind foot (length from heel to tip of longest toe)	Usually over 1 ½ inches (40 mm) Long sixth pad on foot	Generally less than 1 ½ inches (40 mm) Long sixth pad on foot	Generally less than [illegible] inch (20 mm) Round sixth pad on foot
Mammary glands (in female)	Normally 12 (3 pairs on chest 3 pairs towards groin)	Normally 10 (2 pairs on chest 3 pairs towards groin)	Normally 10 (3 pairs on chest 2 pairs towards groin)
Droppings	In groups spindle shaped	Scattered sausage shaped	Scattered fine spindles

frugivorus, which also has a tawny back but has a white or lemon coloured belly [98] However, many intergradations exist between these three sub species and it is the opinion of some experienced observers, e g, Jorge [89] and Webster,[210] that, for the practical purposes of plague work at least, these colour distinctions should be disregarded It is interesting to see that this opinion has been endorsed by Ellerman [46] who now considers *R r alexandrinus* and *R r frugivorus* not as true subspecies, but merely as forms or colour phases of a typical *rattus* race

Distribution

General agreement seems to exist that the three rodent species now under review are of Asiatic descent, the original home of *R norvegicus* and of *M musculus* having been Inner Asia, and that of *R rattus*, Burma and India [73] It is in agreement with this concept that *R norvegicus* is the pre dominant species in north China, where it presumably arrived long ago by a land route, while *R rattus*, which is restricted to the coastal districts appears to be a comparative newcomer In central and south China on the other hand both rat species usually coexist in varying proportions

In contrast to what has been found in north China *R rattus* alone is found in the inland districts of India *R norvegicus* being present in seaports only (Webster, [210] Taylor, [195] George & Webster, [60] George & Timothy [59]) Referring to *R rattus*, Ellerman [46] stated that

"in India wild (whitish bellied) and commensal (dark bellied) races occur together extensively There is doubtless much interbreeding between the two"

In India as in China commensal mice (*M musculus*) belonging to various and, in part not well identified subspecies or races are widely distributed

Regarding the time of arrival of the three species of commensal rodents in Europe it seems to be universally agreed that *M musculus* came from Asia with a people not older than the Neolithic (Hinton [3]) The question as to when the rats penetrated into Europe is on the other hand still debated

Dealing with this problem in 1936 Wu Lien teh [215] felt entitled to refer to the generally accepted opinion that the ships of the returning crusaders were responsible for the importation of these pests (*Rattus rattus*) in the 12th century —an opinion endorsed by several subsequent writers e g Holsendorf [76] Morgan et al [141] Shrewsbury [181] and Tricot Royer [204] However as has been mentioned in chapter 1 in a recently published note MacArthur [114] quoted evidence to show that the rats have been known in Europe since ancient times

While the possibility that *R rattus* have been present in Europe longer than has usually been assumed should be given attention, it is difficult to believe that *R norvegicus* became widespread there at an early date Voicing the opinion of most authorities Hinton [73] stated in this respect that

" the people of western Europe had no knowledge of the species [*R norvegicus*] until 1716 when it was introduced to Copenhagen as the result of a visit by the Russian fleet In the year 1727 a mouse year in the Caspian region vast hordes of these rats according to Pallas moved westwards after an earthquake (but probably in search of food) they swam across the Volga and swarmed into the houses of Astrakan Thence they spread across Russia into western Europe

As a result of this mass immigration *R norvegicus* became the predominant rat species in Europe As maintained by Liston [107] this replacement of *R rattus* by the Norway rat was due not so much to an actual struggle between the two species but to the fact that the former rat could adapt itself less well to changes in the habits of man which led to the expulsion of the rats from human dwellings

The validity of Liston s contention was proved by further observations which showed that *R rattus* was gradually apt to regain more and more of its lost territory In this connexion Jorge [89] pointed not only to the importance of an incessant importation of this species by vessels but also to the environmental changes produced by a further progress of civilization which put the Norway rats at a disadvantage Thus in Great Britain it was found that structural improvements such as impermeable floors ferro-concrete construction and separate sewerage systems were inimical for *R norvegicus* while *R rattus* profited not only by the handicaps of its competitor but also by the availability of fixtures like telephone wires which facilitated its progress from building to building

It has to be noted, however, that the conditions arising during the second World War were once more apt to favour the Norway rats Morgan[140] stated in this connexion that

> as the result of enemy action sewers have been opened up and other barriers against the brown rat have been destroyed It is anticipated therefore that the black rat [*R. rattus*] will have a tough fight to maintain his hold against an invader whose tastes are more catholic and who will live and breed under conditions that are not acceptable to his brother'

With regard to Africa, reference was made in chapter I to the statement of Jorge[90] that *R norvegicus* was preponderant in the Mediterranean part of the continent, while *R rattus* reigned supreme in ' Greater Africa Attention was also drawn to interesting observations in Central Africa according to which *R rattus*, though now forming the principal plague reservoir in Uganda, seemed not to have arrived there before the beginning of the present century Considering the rather conflicting views as to how this introduction took place, Hopkins[8] reached the tentative conclusion

> that *R rattus* was brought into the inland areas of Kenya and into Uganda by the railway which was begun at Mombasa in 1896 and reached Kisumu at the end of 1901

As shown by a recent invasion of *R rattus* into the Kasenyi region of the Belgian Congo, the infiltration of this species into Central Africa is still continuing

The distribution of the two rat species in South Africa seems similar to that noted in India, i e , *R norvegicus* are found in the ports and their immediate vicinity, while *R rattus* are widely distributed in the interior (Davis)[40] *Mus musculus* apparently shows an even wider distribution, as noted earlier, it was the only commensal rodent in parts of the Cape midlands

Dealing with the rat problem in the USA, Holsendorf[6] stated that

> the black rat *Rattus rattus rattus* otherwise known as the English rat or ship rat was introduced to Europe in the twelfth century and was transferred to America about 4 centuries later This antedated the arrival of the brown [Norway] rat by at least 200 years

As noted earlier, Eskey & Haas[48] were of the opinion that the importation of the two species into the coastal areas of the west took place at a time well in excess of 100 years

Most interesting data on the present distribution of the commensal rats in the USA is given in a handbook entitled *Rat borne disease prevention and control*,[93] published by the Communicable Disease Center of the US Public Health Service

Contrary to formerly held beliefs, it is stated in the handbook that like the common mice *R norvegicus* have become established in nearly

all parts of the USA It was noted in particular that though more than 99% of the rats on ships arriving in the USA from abroad were *R rattus* this species predominated in only some of the South Atlantic and Gulf Coast ports in many seaports of these areas Norway rats were preponderant

It appears on the other hand that recently, *R rattus* probably because of its inability to compete with the Norway rats in the southern seaports

" has moved inland more and more each year and now predominates in a number of inland cities How far it will spread is uncertain but it is not likely to become widely distributed in the Northern States as the temperature may limit its spread in that direction " [68]

In the USA as elsewhere changes in local conditions were found apt to bring about shifts in local dominance from one rat species to another

In Canada commensal rats (mainly *R norvegicus*) have been known to exist since the earliest times on the west coast of British Colombia [81] A few places in the interior of this province also became infested e g Nelson where *R rattus* were found The prairie regions seem to have remained free until about 1900 when rats appeared at the international boundary of North Dakota and Manitoba A gradual invasion of Manitoba and Saskatchewan followed so that rats are now established in all the larger municipalities of these two provinces So far however, rats do not seem to have colonized in Alberta [81] Brown [23] stated in this connexion that they were repeatedly imported into this province by the railways but were always found and destroyed

The distribution and comparative importance of the two species of commensal rats in the South American plague areas has been dealt with in chapter 1 Generally speaking *R norvegicus* appears to be the predominant species in the various Latin American countries (Moll & O Leary [139])

General habits of rats and mice

From Hinton s description [3] of the general habits of the commensal rats it may be gathered that

(*a*) both *R norvegicus* and *R rattus* are normally nocturnal in habits spending the day in their nests

(*b*) while establishing their shelters as near as possible to their food supplies they may go further away in search of food When regularly undertaking such foraging trips they usually establish temporary hiding places en route which if convenient may eventually be turned into the permanent dwellings

(*c*) The rats invariably follow definite runs when leaving their shelters proving so conservative in this respect that they may be caught by unbaited traps set in their path

The general habits of the commensal mice are similar to those of the commensal rats. As a rule, however, they move about only in the immediate vicinity of their shelters, going, as maintained by Harrison[69] not further than 10 feet (approximately 3 m) from them. Another important point is that, in contrast to the commensal rats, particularly the Norway rats, *M. musculus* do not display shyness to alterations in their environment ("new object reaction" quoted by Barnett[5]).

Gnawing. Like all rodents, the commensal rats do not gnaw only to get access to food supplies but they must also do so in order to keep their rapidly growing incisors short enough for use.

Describing the gnawing abilities of the rats, the handbook on rat borne disease[208] states that these animals "will gnaw through any material with a gnawing edge and with a degree of hardness less than the hardness of the enamel of their teeth," including many synthetic building materials, unhardened concrete, and even lead pipes.

Burrowing. While *R. rattus* are more proficient in gnawing than the Norway rats, the latter show outstanding prowess in burrowing. However, in India *R. rattus* were seen to dig in soft materials like earthen floors and walls[15] and in the USA were also found able to burrow underground shelters (Milmore,[130] Perolio[151]).

It is important to note that although burrows for shelter and nesting rarely exceed 18 inches (approximately 45 cm) in depth, rats will dig much deeper and further to gain access to food supplies.[208]

Gaining entrance. Rats have been found able to gain entrance through openings admitting a cylinder with a diameter of half an inch (1.27 cm).[11]

Reaching and jumping. As stated in the above-mentioned handbook[208]

"rats have been observed reaching successfully from one point of vantage to another almost as far as their own length along smooth vertical walls. For this reason the figure of 18 inches [approximately 45 cm], the maximum reach of a large rat plus a small allowance for safety, represents the distance which must be completely clear of possible holding points."

It was also noted that

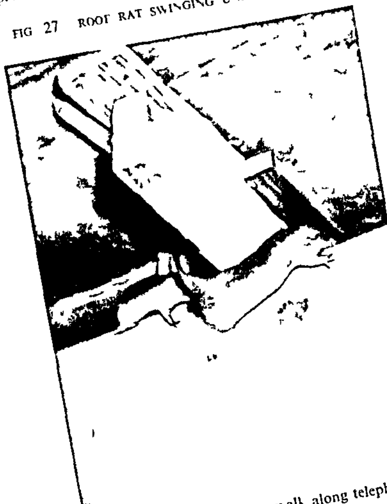
"a rat can be expected to do a standing high jump of nearly 2 feet [approximately 60 cm]. With a running start, and with a trick of bouncing against the vertical surface two-thirds of the way up to gain momentum, the rat can jump 3 feet [approximately 90 cm] and occasionally a little more than 3 feet. Jumping out and down from a standstill, a rat can cover a horizontal distance of about 8 feet [approximately 2.5 m] while dropping less than 15 feet [approximately 4.5 m] and it can do even better with a running start."

Rats have been observed to drop 50 feet (approximately 15 m) without being killed.[11] A roof rat swinging under a rafter is shown in fig. 27.

Climbing. While *R. rattus* is undoubtedly more able and prone to climb than the Norway rat, the latter will climb almost as well as the

former when necessary In fact as recently stressed by Jany [80a] *R norvegicus* by adopting tree climbing habits may become dangerous not only by depredating fruit trees, but also by entering the upper storeys of houses from trees planted too close to the buildings

FIG 27 ROOF RAT SWINGING UNDER A RAFTER



Occasionally Norway rats will also walk along telephone wires as well as *R rattus* do habitually [08]

Rats are able to climb any vertical surface on which they ca toenail hold but do not do so regularly in order not to rub th pads raw [08] However they can easily climb vertical wires which rat guarded and vertical pipes if the diameter of the latter does n 3 inches (approximately 7 5 cm) they can also climb on the o any vertical pipe which is within 3 inches of a wall or other c support Likewise they can climb on the inside of vertical pi ...ded with a diameter ranging from 1½ to 4 inche

Within the limits imposed by their size, the commensal mice climb well It may be added that these agile animals are also proficient in jumping and burrowing

Swimming As shown by many observations, the Norway rats are excellent swimmers and divers They are able to "swim through the water seals in toilets and floor drains without fear or hesitation'[208] and habitually infest sewers and drains, the more so because—being by no means dainty in feeding habits—they find there food and drinking water[15] Likewise, they may establish their shelters in the banks of surface waters in order to prey on fish food or even young fish (Cottam[30])

R rattus and *M musculus* do not enter water voluntarily However, resembling the proverbial sailor who though spending his life at sea has never learnt to swim, *R rattus* is the species of commensal rat usually found on ships

Feeding habits and food requirements

As has been generally observed, and has been confirmed by baiting tests with Norway rats,[97] owing to their nocturnal habits the commensal rats and mice usually feed even in a quiet environment, at night However, if their food supply is short, they may snatch some nourishment at any time of the day, and may then devour the food available at the place of discovery instead of carrying it back to their shelters for consumption or storage, as they usually do

Recent observations by Calhoun[6] seem to suggest that rats differ in social standing within their colonies, each colony having a domineering bully which partakes first of the best food available and lays in no stores in contrast to the weaker rats which try to do so whenever they have a chance

As previously noted, *R rattus* and also the commensal mice being dainty feeders, are rather dependent on food supplies destined for human consumption while the Norway rats may subsist on any nourishment available to them on garbage heaps, in sewers, and the like

No doubt can exist that if given a choice, commensal rodents will show marked preferences for certain kinds of food, but they can adapt themselves to the staple foods available, in particular often living mainly on cereals It is interesting to note in this connexion that, Davis[33] on the basis of studies at Baltimore Md maintained that

> Norway rats must have a substantial amount of grain (usually available in the form of bread) in their diet Rats will starve in the midst of plenty of raw or cooked vegetables"

While little credence can be given to many of the statements made in regard to the amount of food a commensal rodent can consume in a given period of time, some reliable figures have recently become available

Thus Chitty & Shorten estimated that on an average a Norway rat consumed 30 g of wheat per day (0 35 g to 0 50 g per gulp) An estimate made in India [81] was that *R rattus* consumed about 3,908 g of rice per year or 10 7 g per day—a figure which tallies well with the 11 g of wheat found by McDougall [117] to represent the daily food consumption of *R conatus*

Water requirements

Though *R norvegicus* has been found able to subsist under laboratory conditions for quite considerable periods without water in general water is indispensable to these rats as they are used to drinking freely If neces sary they may climb the roofs of houses to get to water tanks

R rattus appears to be far less in need of drinking water supplies than the Norway rat Still according to the above mentioned observations in India [81] *R rattus* consumed 143 5 ounces (approximately 4 kg) of water per year or 0 4 ounce (approximately 11 g) per day It was found that this species could subsist on dry rice without water up to 24 days and on water alone for 10 days while controls given neither food nor water subsisted up to 6 days

Harbourage

As emphasized in the handbook on rat borne disease [208] the possibilities for the harbourage of commensal rodents are almost endless in variety The animals may prepare shelters by burrowing in embankments near or under buildings or may settle in the latter where as stated by Holsendorf [5, 6] three types of harbourage are available

(1) *structural*—offered by permanently protected spaces between double walls, under double floors above false ceilings behind enclosed stairways raised platforms or boxed in pipes etc

(2) *incidental*—offered by furniture and equipment

(3) *temporary*—offered by

"mass storage of material or merchandise rubbish heaps old furniture odds and ends piled in cellars attics and closets and similar accumulations which if left undisturbed for periods of several weeks can and will be used by rats for homes and breeding places" [75]

Commensal rodents are apt to harbour not only in or near premises inhabited by man but also in those parts of houses or in outhouses where domestic animals are kept in buildings where foodstuffs are processed or stored and in establishments such as slaughterhouses

Morgan et al [131] found colonies of *R norvegicus* and *M musculus* flourishing in cold storage plants where these animals had adapted them selves to an existence at 17°F (—8 3°C) by growing slightly thicker furs and layers of fat underneath The ability of *M musculus* to live and breed in cold storage plants was confirmed by Laurie [100]

As indicated by the common names often given to them, the "sewer rat", *R norvegicus*, as a rule prefers to live underground, whereas the "roof rat", *R rattus*, harbours in or under the roofs of buildings or in the upper storeys

The Norway rat is well suited for an underground existence, not only because it is much more hardy and far less dainty in habits and food requirements than *R rattus*, but also because, though not keen to climb as a rule, it is outstandingly proficient in burrowing shelters One must also suppose that, in hot climates, this rat prefers the comparative coolness of its burrow to an existence above ground

The reasons why *R rattus*, an excellent climber, prefers to shelter in the upper parts of buildings, appear to be threefold it is less able to withstand hardships, particularly a cold environment, than the Norway rat it is much cleaner in habits and feeding than *R norvegicus*, by harbouring high up, it is able to keep out of the way of any Norway rats living in the same premises underground, or on the ground floors

The importance of the last mentioned factor is proved by observations which have shown that, in the absence of Norway rats *R rattus* may live on the ground floors or even underground For instance according to Girard,[63] in Madagascar, where Norway rats are absent *R rattus* inhabits the sewers in their place

If the two rat species coexist in a locality, their relative distribution may be irregular Thus Milmore[135] noted that, under such circumstances sometimes buildings or whole city blocks were infested by *R rattus* and *M musculus* only whereas adjacent houses or blocks also harboured *R norvegicus*

Breeding habits

The commensal rats and mice are extremely fertile They reach sexual maturity long before they have completed their growth The classical investigations undertaken in this respect by the Plague Research Commission in Bombay (quoted by Hinton[73]) yielded the following results

	Minimum weight of sexually mature animals		*Average weight of adult animals*	
	(g)	*(ounces)*	*(g)*	*(ounces)*
R norvegicus	100	about 3 5	250	about 9
R rattus	70	about 2 5	140	about 5

As confirmed by the recent investigations of Davis & Hall[38] male rats remain fertile throughout the year According to Hinton[73] the females have a sexual season extending for any one female for 9 months of the year, during which time they come "on heat" at intervals of about 10 days These periods last for a few hours only and if no copulation takes place during that time, a female cannot be impregnated until her next heat During

the sexual season some evidence of the regular occurrence of heat is shown even by a pregnant female parturition during the season is immediately followed by heat so that a female may be impregnated a few hours after the birth of a litter

The period of gestation is usually about 21 days but may be prolonged for a further 10 days in a female nursing her young

According to figures supplied by the Plague Research Commission (quoted by Wu Lien teh[21]) the average and maximum numbers of young rats per litter were

	R norvegicus	*R rattus*
average	8 1[f]	5 2
maximum	14 0[g]	9 0

The menopause usually appears at the age of 15 to 18 months but particularly good environmental conditions tend to delay its appearance[3]

Dealing with the breeding habits of *M musculus* Hinton[73] stated that these animals

attain sexual maturity when three months old The sexual season of the females is a very long one The period of gestation is normally from nineteen to twenty-one days it may in certain circumstances be shortened to twelve or thirteen days Heat not lasting longer than twelve hours rapidly succeeds parturition Many litters are born throughout the year but fewer in the cold months The number of young per litter is between five and six but it may be as many as nine or as few as two "

Scrutinizing the evidence available in regard to annual pregnancy and lactation rates both from cities in the USA and other places e g Bombay and Dakar Davis[33] arrived at the following figures for *R norvegicus*

pregnancies per year	4 3
lactation periods per year	3 8

However since there was a great loss of sucklings Davis reached the conclusion that an adult female weans rats at the rate of about 10 rats per year

It is important to note that this figure is rather below the estimates given by earlier plague workers in regard to the number of young rats produced per year

Though rat breeding continues throughout the year particularly under optimal environmental conditions it has been observed that as a rule there are one or two seasons during which the number of pregnant and nursing females is maximal Studying this problem Buxton[1] concluded that in temperate and subtemperate climates the main breeding periods coincided with the warm season That great heat exerts an unfavourable

f 8 7 according to D s

g According to Holsendorf litte f more th n 20 ha e been obs rved

influence on the breeding rate seems to be suggested by the observation of Raynal [167] that, at Shanghai, which has an excessively hot summer, the breeding season of the rats coincided with the end of winter and the beginning of spring

Buxton [24] was of the opinion that the degree to which the rats breed is mainly determined by the state of their nutrition Their breeding rate might, therefore, be influenced by an abundant or poor harvest which in turn, is governed by climatic conditions

It is quite likely, therefore, that in the case of the rats, as in that of the wild rodents, a mechanism regulating their numbers according to Elton's concept [47] might be at work There can be no doubt, however, that in the case of the rats, many extrinsic influences would be bound to interfere with the operation of this "natural regulatory mechanism

Density of rat populations

One cannot doubt that a relation exists between the density of the rat population and that of the human population Thus, it was recently pointed out by Gracie [66] that in Great Britain the *greatest concentrations of rats in* sewers were present beneath the most densely populated precincts

At the same time, however, one should give no credence to the time worn statement that the number of rats in a given locality invariably equals that of the human population—an estimate which, as pointed out by Davis & Fales,[35] was originally derived from statements regarding the rat incidence on English farms Davis & Fales announced as the result of their observations in Baltimore Md that, in 1947, the estimated total number of rats in Baltimore was 200 000, or not more than 1 rat per 5 human inhabitants They added however, that this figure was much lower than that of 400,000 rats estimated to have been present in Baltimore in 1944 and ascribed this reduction in the rodent population on the one hand to rat control measures and general sanitary improvements, and to an influence of the cold, wet spring of 1947 on the other

Obviously, therefore, it is impossible to set standards for the density of rat populations in different places or at different times in any one place On the contrary, it is necessary to ascertain in each instance the number of rats present through a preliminary survey and then to watch the trend of the rodent population with the aid of the methods which will be described in a future chapter

As far as one is entitled to draw conclusions from the scanty *information* available, it would seem likely that in other well sanitated cities, as in Baltimore less than one rat per head of human population would be found There is reason to assume that in less well sanitated cities and in smaller settlements in general, the number of rats equals or even exceeds the number of people This is presumably the rule on farms (Tice [198])

Population dynamics

In a profound study on the characteristics of global rat populations Davis[33] drew a distinction between two types of changes in rat populations

(1) an increase up to the capacity of the area in question to support the rats and

(2) fluctuations due to changes in this capacity

As pointed out by Davis[33] in the latter connexion the rat population in a place at any given moment is the result of an interaction of two factors —reproduction and mortality The effect of a possible third factor—namely the increase or decrease of the rodent population through movements—is actually of no importance

With regard to the force of mortality, Davis[32] maintained that, as indicated by data from a farm only about 5 out of 100 rats lived for 12 months the average life of a rat in a population being about 6 months

Dealing in greater detail with the quantitative relations of the various regulatory factors Davis[33] stated the following

(*a*) *Environment* A shortage of either food supplies or nesting sites (harbourage) limits the number of rats in a given locality or at a given time This limitation is density independent i e it is operative regardless of the size of the rat population in question

(*b*) *Predation* Davis used the term predation to indicate all causes of rat mortality including that caused by cats viruses traps or poisons He emphasized that in contrast to the above mentioned environmental factors predation was density dependent the proportional effect exerted by predators decreasing pari passu with a decrease in the rodent population according to the law of diminishing returns

(*c*) *Competition* As is obvious the effect of competition among the rodents for food living space and mates is also density dependent increasing hand in hand with population increases

Comparing the practical value of predation with that of sanitation which increases competition between the rodents for food and living space Davis came to the important conclusion that while it is generally feasible to increase competition it is rarely possible to increase predation to a satisfactorily high degree

Movements and migrations

As in the case of wild rodents when dealing with the movements and migrations of the commensal rodents it is necessary to distinguish between

(*a*) short range daily (or one should rather say nightly) excursions necessitated by the search for food and water

influence on the breeding rate seems to be suggested by the observation of Raynal[167] that, at Shanghai which has an excessively hot summer the breeding season of the rats coincided with the end of winter and the beginning of spring

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Comparing the practical value of predation with that of sanitation which increases competition between the rodents for food and living space Davis came to the important conclusion that while it is generally feasible to increase competition it is rarely possible to increase predation to a satisfactorily high degree

Movements and migrations

As in the case of wild rodents when dealing with the movements and migrations of the commensal rodents it is necessary to distinguish between

(*a*) short range daily (or one should rather say nightly) excursions necessitated by the search for food and water

(*b*) seasonal or, as Macchiavello[119] called them, 'reversible migrations undertaken periodically to fields under cultivation, and return to the settlements after the harvests have been gathered

(*c*) true, progressive migrations covering considerable distances

Recent observations have confirmed that the urban rats and mice spend most of their life within a very limited home range Davis,[33] summarizing the evidence available in this respect, maintained that movements of rats even from one street block to another were rare, and that if a few rats left their home range, the chances of their survival were slight

Commensal rodents living in rural environments may also have a strictly limited home range[31, 63] but, as noted above, they are able to make seasonal migrations

Some observers have noted that commensal rats living in rural environments are also apt to undertake progressive migrations covering wide distances Recently, Macchiavello[119] stated that these animals may move en masse over hundreds of kilometres, possibly in search of vitamin E in grain germs

Nevertheless it would seem that, in most plague areas, such mass migrations are exceptional It is true, as discussed earlier, that even now, commensal rats invade areas previously unoccupied by them, but *it is probable that these movements are not mass migrations in the true sense*, but rather gradual infiltrations

Passive transportation

As discussed in chapter 1, a passive transport of rats by ships and railways was of most fateful importance for the spread of plague Further there can be no doubt that in addition to rail transport in some plague areas, motor vehicle transport—particularly trucks—led to a spread of the infection by the carriage not only of infected fleas, but also of infected rats

It is less certain whether this holds generally true of primitive means of transport Summarizing the evidence available in this respect, the annual report for 1937 of the Eastern Bureau of the League of Nations Health Organisation Singapore, pointed out that observations made in the Cumbum Valley of south India did not support the view that rodents or even fleas were frequently carried in consignments of rice transported on *bullock* carts It was noted, however, that, in the opinion of some observers even pack horses are common means in Java of conveying infected rats and their fleas[10-]

It is curious to note that, according to a report made in 1942[181] a live mouse was found in the galley of an aeroplane arriving at Miami Florida, from Puerto Rico It was stated in this connexion that

> considering the increase in the size of transport planes the carrying of foodstuffs that are attractive for rats and the ingenuity of these animals in boarding vessels seeking

food supplies establishing nesting places and avoiding man s devices for destroying them the possibility of rats boarding airplanes is certainly not remote "

Damage caused

Calling the rat Public Enemy No 1 among the animal pests Holsendorf [6] aptly quoted a statement by Creel & Akin [31] who considered this rodent not only as the least useful but also as one of the most danger ous of nature s parasitic animals living at man s expense

A recently published indictment of the commensal rats stated that apart from their ominous role as the reservoirs of plague and other diseases they caused tremendous harm by consuming or spoiling grain merchandise fruits vegetables nuts and eggs by killing chicks and other young birds as well as young pigs and sheep by damaging sugar and other plantations, and by starting fires by gnawing the insulations on electrical conductors and in other ways

Though many authors have tried to assess the monetary equivalent of the depredations caused by the commensal rodents one must fully agree with Kalmbach s statement that hardly any reliable data is available in this respect [93] This holds particularly true in the case of foodstuffs because as has been pointed out with much reason [14] rats will feed on any edible substance from valueless garbage to expensive packaged products which they may damage to an extent far in excess of the value of the amount of food they actually consume

That the damage done by rats to foodstuffs may far exceed the loss caused by actual consumption has been clearly demonstrated by the recent experiments of Barnett [6] Forty rats were divided into 4 groups of 10 and each group was put into a separate room One ton of wheat divided into 9 sacks was placed in each of the 4 rooms and left for 12 20 23 and 28 weeks respectively The rats fouled 70 4% of the wheat while causing a weight loss of only 4 4% The total monetary loss amounted to 18 23% of the value of the wheat and sacks most of this was due to the damage done to the sacks

With regard to the diseases caused by the commensal rodents it has been recently stated by Mohr [138] that these animals are responsible or probably responsible for the following infections or infestations

Disease	Reservoir	Mode of transmission to man
Plague and murine typhus	Rats and to a much lesser extent *Mus musculus*	Through rat fleas
Salmonellosis	*M musculus* and rats	Contamination of foodstuffs by droppings and possibly also through rat fleas

Disease	Reservoir	Mode of transmission to man
Leptospiral jaundice	Norway rats in particular	Contamination of food and w by urine of infected animals
Rat bite fever	Rats and probably also M musculus	Bite by an infected rodent
Rickettsialpox	M musculus	Through mouse mite (Allodermanys sus sanguineus)
Lymphocytic choriomeningitis	Probably M musculus	Possibly through direct contact or through contaminated food
Amoebiasis and tapeworm infections	Rats	Food contaminated by droppings
Histoplasmosis	Dogs and possibly Norway rats	As stated by Mohr both rats and human beings might acquire infection from a common source but rats might take a share in infecting man

Plague in Commensal Rodents

As shown by the studies of Abel,[1] Tiraboschi [203] and others the now generally accepted concept that, as a rule plague epizootics in the commensal rats precede and cause human manifestations of the disease had been arrived at by some early observers Thus a sacred, poetical work, the *Bhagavata Purana*, written centuries ago in Sanskrit warned house holders in Hindustan to leave their homes as soon as rats fell from the roofs and died [107] To judge from a statement recorded by Forbes [5] (quoted by Simpson [186]) this advice was still followed by the population of Marwar during the Pali plague of 1836 8

A great rat mortality, preceding and accompanying the human outbreaks, was noted by a British official during the Kumaon outbreak of 1834 5 The same phenomenon was observed and commented on by medical men during several of the later epidemics in that area and during earlier outbreaks in Garhwal (Simpson [186])

The Chinese in Yunnan were also well aware of the fact that rat mortality presaged outbreaks of human plague [15] Thus during the epidemic at Chaochow in 1792, Shih Tao nan (quoted by Wu Lien teh [215]) in a poem entitled Death of rats which he wrote a few days before he died of the disease deplored that

> Few days following the death of the rats
> Men pass away like falling walls !

In China also some medical men appear to have suspected that the rats were of etiological importance in plague Lowry,[111] noted that during the 1882 outbreak at Pakhoi in nearly every house where the disease broke out, the rats had been coming out of their holes and dying on the

floors and dissected some of these animals but found nothing definite microscopically.

Niles[147] recorded that, during the 1894 outbreak in Canton, one of the Chinese officials urged the population to kill rats and to collect dead ones, offering to pay, out of his own pocket, 10 cash for every carcass brought to him.

Rennie[168] who examined a considerable number of the rats thus collected noted that 90% of them showed enlargement of lymph nodes which, however, was present "in a much less marked degree than in the human subject", and he raised the question "is the disease in man and animals identical?"

In marked contrast to the advanced views held in the East, no convincing evidence seems to be available to show that the role played by the rats in the causation of plague outbreaks was realized at an early date in Europe. Though it was commonly believed that animals as well as humans were affected by the plague poison which was supposed to be fermenting in the ground, no particular attention or even none at all was paid in this connexion to the rats (MacArthur[113]). It is also significant that the rats and mice often depicted in illustrated texts and in pictures after A.D. 1250 invariably seem to be healthy (Neustatter[117]).

One of the main reasons why the rats attracted no attention was probably that their dying seemed of no importance in comparison with the havoc caused by simultaneous "pestilences" among domestic animals which, though actually due to other infectious agents, were then thought to be identical with plague.

Far more distressing than this early ignorance is the fact that, even when evidence proving the existence and importance of rat plague became available about sixty years ago, it was not accepted forthwith.

It should be noted in this connexion that Yersin (quoted by Lagrange[94]) obtained positive findings in rats almost immediately after the discovery of the plague bacillus in June 1894, for he noted in his diary under the date of 23 June 1894: "I search and find the organism in the corpses of dead rats, and there are many throughout the city."

Yersin amplified this statement in the first elaborate report on his work at Hong Kong,[17] reaching the conclusion that "plague is therefore a contagious and inoculable disease. It is probable that the rats are the principal vector."[a]

Convincing as Yersin's evidence was, it did not impress the other workers at Hong Kong. Lowson's official report on the 1894 epidemic,[11] for instance, contained the following statement: "The question of the infection of rats previous to the epidemic being noted in human beings has been made too much of."

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[a] La peste est donc une m[illegible]die [illegible]tagieuse et [illegible] Il est pr[illegible] [illegible] les [illegible] le principal [illegible]

Since, as Millot Severn [178] (one of Lowson's successors) put it, the early plague investigators in Hong Kong were "obsessed with the idea that bubonic plague was primarily a gastro intestinal infection , it was not until 1901 that rat destruction was started there (Brown [20]), even though this method had been expressly recommended by Yersin [18] as early as 1897

Similarly, as recently stated by Link [106] in an article entitled Plague on the high seas', the importance of the rats in ship borne plague was not recognized until 1906, even though, between the years 1900 and 1904, infected animals had been found on vessels on nine different occasions

Nevertheless, Yersin s findings were confirmed by some early plague workers, among whom Ogata [149] in Formosa (1897) Simond [185] and Hankin [68] in India (1898), Ashburton Thompson [196] in Sydney (1900) and Blackmore in Port Elizabeth (1902) were particularly praised by Liston [107] who, himself, played a worthy part in plague research In 1905 the British Secretary of State for India following proposals made by the Lister Institute a year earlier established a Joint Advisory Committee, appointed jointly by him, the Royal Society, and the Lister Institute The Committee appointed a Plague Research Commission and, from 1905 onwards, Liston and other workers (notably, Lamb, Petrie, and Rowland) took part in its activities The findings of this Commission formed an outstanding monument to scientific teamwork and were instrumental in defining the fundamental problems of plague in rats as well as in rat fleas [15]

As summarized by Lamb,[98] at the time the Plague Research Commission commenced its work, even those who were agreed upon the causal role of the rats differed in their views as to how the infection was spread among these animals Some observers were still influenced by time honoured beliefs ascribing importance to an infection through the air, through direct contact, or through contaminated inanimate objects (fomites) Others laying stress upon the cannibalistic habits attributed to rats, considered that plague might be transmitted through the rats feeding on one another Through a series of well planned experiments the Commission was able to refute these beliefs and, at the same time to establish that plague was principally an insect borne—and particularly, a flea borne—infection

Relative importance of roles of rats and mice

Rats In the past, as well as recently, it has been argued by some writers that *R norvegicus* is less susceptible to infection with *P pestis* than *R rattus*, and therefore cannot independently act as a plague reservoir

However no convincing evidence has ever been brought forward to support such claims Reliable laboratory investigations have proved that both species are equally susceptible to the infection and as pointed out earlier in this monograph, observations made in the various plague areas have

shown that each of the species is capable of taking an independent part in the causation and perpetuation of the disease

At the same time it must be realized that if coexistent in a plague affected locality, the two rat species may play different roles This point was well illustrated by the investigations made by the Plague Research Commission in Bombay[98]

The Commission s observations showed that as far as could be judged from statistical evidence *R norvegicus* though considerably less common in India, appeared to be twice as liable to natural plague infection as *R rattus* There seemed no doubt however that this higher incidence of the disease in the Norway rats was the result not of a higher susceptibility to the infection, but of their far heavier flea infestation Evidently it was mainly due to the same reason that *R norvegicus* was of prime importance for the carry over of the infection during the inter epizootic periods

It was further found that at the onset of the plague season the infection became rampant among this species first Then after an interval of about ten days epizootics appeared among *R rattus* and these in turn were mainly responsible for the appearance of human plague after a further interval of 10 to 14 days However it was certain that this evolution was due merely to the fact that *R rattus* lived in houses and therefore in close contact with man whereas *R norvegicus* though infesting the ground floors of houses were more numerous in other locations such as gullies com pounds stables warehouses and shops

The conclusion reached by the Plague Research Commission that differ ences in the habitat of the rats and in their flea infestation rather than differences in their susceptibility to the infection determined the role played by these animals in plague has been endorsed by other workers recently by Roberts[169, 1 1] in Kenya Though *R rattus* alone were involved in the Kenya outbreaks two types of plague manifestations could be dis tinguished—namely an urban one characterized by the occurrence of epi demics and a rural one of an endemic type In the opinion of Roberts it was of importance in this connexion that in the urban localities the rats lived underground so that both they and their fleas were apt to come into close contact with man whereas in the rural areas there was little if any possibility for such a close contact because the rats there lived in the thatch of roofs In fact as maintained by Cormack[49] and Roberts[1 1] the roof rats did not enter the interior of human dwellings at all during the day they hid in the thatch and emerged at night on the outside of the huts and then proceeded to the cereal stores to feed

Mice As pointed out by Jorge[69] *M musculus* usually lives in close contact with man and would be rather dangerous were its role in plague similar to that of the rat Actually however though often found infected during the course of rat epizootics the mouse is usually a victim of the

disease rather than an agent in its perpetuation The main reasons for this fortunate circumstance are

(a) as established by the Plague Research Commission and confirmed by other workers, these animals were often less susceptible to plague infection than the rats collected in the same localities,

(b) the flea index of the mice was often considerably lower than that of the rats and, still more important the specific flea of the mice, *Leptopsylla segnis*, is not an efficient plague vector and hardly ever attacks man

However, as shown by several observations, *M musculus*, if infested by other than its usual fleas may be of some, or even of considerable importance in the perpetuation and spread of plague Mention has already been made of the role ascribed to these animals in south-east Russia, and also of the observation by Herivaux & Toumanoff[7] in Indochina of an epizootic among *M musculus* infested with *X cheopis* Some importance was also ascribed to this rodent species in Brazil[13] Girard[63] while stating that the mice usually did not appear to be involved in the plague outbreaks of Madagascar referred to one instance where in a rural environment a considerable epizootic had been found present among these animals as well as among the rats

Spread of rat epizootics

It is obvious that when paying attention to the spread of plague not from one individual rat to another, but from one group of these animals to other groups, a distinction has to be made between what Gill[8] adequately called the intramural spread of the disease from one house, block or part of a settlement to adjacent houses, blocks or precincts, and spread at distance or, as observers in Java have called it, ' metastatic spread

It is easy to understand how plague, conveyed either by the rats themselves or by their fleas, may creep from one house or group of houses to others, the more so since, as observed by the Plague Research Commission these rodents may desert infected buildings particularly when the human inhabitants have died or have left

Such a spread of the infection may be extremely slow, one instance was recorded where a rat epizootic in India took six weeks to travel 300 feet (approximately 91 m)[15] A further fact of great importance is that the intramural spread of plague often takes place in an irregular manner, leaving the rat population of some houses blocks and even whole precincts unaffected

The question as to whether active movements of the rats are responsible for the long distance spread of plague and if so to what extent has been the subject of considerable debate In the opinion of the Plague Research Commission[98] this factor was of no importance the spread of the infection

per saltum being effected by the passive transportation of rats or their fleas by human and goods traffic This opinion has since been shared by most subsequent workers

Factors limiting the spread of rat plague

It may be maintained that the spread of plague among the commensal rodents is limited mainly by

(*a*) factors governing the role of the insect vectors particularly the fleas

(*b*) a diminution in the number of rats by severe epizootics which reduces the chances of infection among the scattered survivors

(*c*) the irregular spread of the infection which may by pass groups of animals living in the vicinity of plague foci

(*d*) a state of non susceptibility to the infection developing in individual animals or in rodent herds which have been exposed to plague

With regard to the last mentioned factor the only one which can be evaluated at present—it should be noted that some writers stressed the importance of an immunity acquired by the commensal rodents in the course of plague epizootics They claimed that the animals became immune by surviving an attack of the disease and sometimes even maintained that rats may acquire an active immunity by exposure to subinfective doses of *P pestis* without passing through a stage of manifest illness More important still these writers postulated that the immunity acquired by the commensal rodents through exposure to plague was passed on to their offspring

While no doubt can exist that commensal rodents particularly those which have recovered from an attack of plague may become immune against the infection it is probable that such an active immunity is maintained for short periods only Gill[6] was of the opinion that the immunity did not last more than a few months

The rather unlikely assumption that the acquired immunity of commensal rodents is passed on to their offspring has been definitely disproved by investigations made by Sokhey & Chitre[180, 182] Experimenting with the offspring of white mice which had survived plague infection owing to having been previously immunized with Haffkine vaccine these workers demonstrated that the young mice when inoculated with standard infective doses of *P pestis* at an age of 3 to 4 months showed no evidence of immunity

For these reasons it would appear that the immunity acquired by some commensal rodents in the course of plague epizootics can play no important and certainly no permanent role in the limitation of the

epizootics However, it is of the utmost importance in this respect that as first shown by the Plague Research Commission[156] and confirmed by other observers, particularly Sokhey & Chitre,[188 189] the presence of plague in a herd of commensal rodents leads to a gradual extinction of the susceptible strains of the animals, whereas resistant strains survive and produce offspring which are also plague resistant

In this connexion the Plague Research Commission[158] established that

(*a*) a considerable number of the Bombay rats were resistant to laboratory infection with *P pestis* produced either through flea bites or through subcutaneous injection of infective doses standardized according to the *rather unsatisfactory methods then available*,

(*b*) the survivors of experimental epizootics were also highly resistant when challenged with such test doses,

(*c*) *rats caught in plague free localities in India showed a high* susceptibility to the infection, amounting to 97% to 100% in the case of Madras City, whereas rats from plague affected localities were more or less resistant, the degree of their resistance to the test doses generally being proportional to the extent to which the places in question had suffered from the disease

Sokhey & Chitre[188 189] repeated these investigations with accurately standardized test doses They compared the aggregate human plague death rate (calculated per thousand of the population) of numerous Indian cities and towns during the period 1899 1929 with the percentage death rate from plague in batches of *R rattus* obtained *from the same localities* As a rule, about 50 adult animals were taken from each locality The results showed that, on the whole, the percentage rates of infection were inversely proportional to the human plague death rates i e the resistance of the rats to test infection was higher the more the locality in question had suffered from plague

Confirming some earlier observations, e g, those of Spencer[191] at Alabama, Sokhey & Chitre also established the *important fact* that even in localities which had never suffered from plague a considerable minority of the rat populations could be resistant to the infection It is therefore obvious that, normally, both plague susceptible and plague resistant rat strains exist side by side, the occurrence of plague upsetting the balance between the two groups by killing the susceptible animals

The question of whether this also holds true of the rat populations in rural endemic areas—only urban *settlements* were investigated by the above mentioned workers—seems thus far, to have received insufficient attention Rao[166] stated that he had found evidence of resistance to the infection in a group of 22 rats caught in a village of Hyderabad State However, George & Webster[60] established the reverse in *the Cumbum*

Valley and similarly Roberts[169] reported that he had found no evidence of plague resistance among the rats of a rural endemic area in Kenya. It would be desirable to confirm these findings through further and more extensive investigations. As far as evidence is available it would appear that the rat populations in rural endemic areas may remain susceptible to infection with *P. pestis*.

Considerable time may elapse before the preponderance of resistant rats becomes sufficiently marked to exert an influence on the plague situation in the localities concerned. For, as summarized by Wu Lien-teh,[21] this process of selection

"may show an indefinite variety in different localities and certain influences may tend to retard it. Liston, for instance, pointed out with reason that an epizootic may leave susceptible rat colonies untouched so that at its termination both resistant and susceptible rats may survive and interbreed. A similar situation may be created if susceptible rodents are imported into a locality where the local rats have become more or less resistant. In fact the Plague Research Commission found that town rats in Bombay showed a higher immunity to plague infection by feeding and by flea transmission than ship-rats from the harbour, which latter had presumably not been exposed to infection."

No doubt can exist, however, that when reaching a sufficiently high degree the resistance of the commensal rodents to the infection is apt to exert a profound influence on the plague situation in the areas concerned. Liston[10] maintained in this connexion that

"the evolution of an immune race of rats following on a long series of epidemics may explain the gaps in the continuity of epidemics which are known to have occurred in the history of plague in different countries"

and that this may also

"afford an explanation for the cessation of epidemics of plague in countries in which changes in the social life of the inhabitants fail to supply us with an adequate solution."

A point of great interest and importance is how long the resistance becoming manifest in the rat populations of infected localities will persist in the absence of plague. The Plague Research Commission (quoted by Wu Lien-teh[15]) noted in this respect that the rats of Vaniyambadi, which had suffered severely from plague in 1901-3, were highly susceptible when tested eight years later. Further information on this point was furnished by Sokhey & Menezes[180] (see table XVIII).

Table XVIII shows that apparently the herd susceptibility to plague became nil in the second year during which rat infection was absent from Bombay but began to rise again in the following year. However, as shown by table XVI (see p. 274), rat plague continued to be practically absent from Bombay.

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numerous suspicious bipolar stained bacilli The diagnosis of plague was supported by the positive results obtained through inoculating white rats with the pooled fleas (*Ctenocephalides canis*) of the dog Fleas (*Ct felis*) from a cat which had died in the same house soon after the dog likewise proved positive for plague

It would therefore seem that, on rare occasions dogs may contract plague under natural conditions Certainly, however, instances of this kind are of little importance when contrasted with the far more dangerous role dogs are apt to play by picking up plague infected rodent fleas and conveying them to their masters

Cats

While experience gained in all major plague areas has confirmed that cats may suffer from plague under natural conditions, it has been much debated whether they can contract infection by feeding, and whether their infection occurs frequently enough to be of importance for the spread of the disease

Araujo [3] and Henriques [71] who recently devoted attention to the former problem, confirmed the conclusion previously reached by Dujardin Beaumetz (quoted by Henriques [71]) that, under natural conditions, cats could contract infection by feeding only if their buccal or intestinal mucosa was traumatized by bone fragments

In the opinion of several observers e g, Henriques [71] and Silva & Valença,[184] cats were not, in general very susceptible to experimental infection with *P pestis* Uriarte & Morales Villazon [07] found that such infection produced a chronic disease characterized by emaciation and abscess formation, but that the causative organisms could not be recovered at autopsy As shown by Henriques,[71] the death of experimentally infected cats could be due to the action of the endotoxin of *P pestis* and not to a bacteraemia

According to Moll & O Leary,[139] plague workers in Argentina often noted instances where the presence of plague in cats seemed to be respon sible for human cases Pozzo,[1 7 158] while admitting that in some parts of the country a high mortality among these animals was observed during plague outbreaks, was not certain whether this was invariably due to infec tion with *P pestis*

In Brazil, the frequency of a rapidly spreading and acutely fatal disease among cats was noted in the plague foci, and sometimes also in the plague free areas (Silva) [18] Bezerra Coutinho & Macchiavello who studied this problem closely, ascribed this fatal cat disease which they called "adeno myelo enterosis ", to infection with a filterable virus [16 115 116 1 1 12 1-3 1 1] This conclusion was questioned by Silva [183] who claimed that the cat morta lity was due, in part to plague

Be this as it may generally speaking the incidence of plague among cats was not high enough to play a conspicuous role in the spread of the infection to man However the cats like the dogs are apt to be dangerous by bringing *infected rodent fleas into the houses*

Shrews

The shrews which live side by side with the rats and regularly visit houses at night play a not inconsiderable role in the spread of plague As shown in Annex 1 table IV (see p 636) they have been found liable to contract natural plague in several plague areas More important still being infested with rat ectoparasites they are apt to convey infected fleas to human habitations

Sharif & Narasimham [1,9] also stressed that *Suncus murinus* besides wandering from house to house could undertake excursions from village to village

Plague-Insusceptible Beasts and Birds of Prey

Ample evidence exists to show that carnivora and birds of prey may play a role in the spread of plague even though they are resistant to the infection because they are apt to pick up and later to disperse infected rodent fleas Some workers have also stressed the fact that birds of prey regurgitate the indigestible portions of their meals some time after they have fed and have postulated that these casts if consisting of the remnants of plague infected carcasses may be a means of conveying the infection to rodents eating such morsels Egorov [41] who seems to have been the first to propound this view found that virulent plague bacilli were present in casts regurgitated two days after an infectious meal by an eagle (*Aquila hipalensis*) which had been fed in the laboratory with the carcasses of plague infected guinea pigs

Experiments performed by Araujo [2] did not lend support to Egorov s contention because it was not possible to demonstrate the presence of *P pestis* in the contents of the gizzards and intestines of buzzards (*Urubus*) which had been fed with plague infected materials However, Jellison [87, 88] repeatedly succeeded in isolating plague bacilli from the casts of owls and falcons which had been infected in this manner In the course of field investigations he was able also to demonstrate several times the presence of *P pestis* in the remnants of ground squirrels which had been partly eaten by birds of prey sometimes after the latter had transported the rodent carcasses for some distance Believing that the ground squirrels were often carnivorous Jellison postulated that they could contract plague by feeding on remnants of infected carcasses

Even if one is ready to subscribe to such views it is likely that infections caused by the consumption of casts or remnants of plague carcasses are of

less importance than those due to the transport of infected fleas by birds of prey

Sergeev,[177] a worker in south east Russia, noted in the latter connexion that many species of birds were apt to seek temporary refuge in the burrows of wild rodents, and that some nested there permanently On the other hand, one species (*Oenanthe oenanthe*), which had been proved to harbour wild rodent fleas known to be plague vectors, nested in houses, and therefore seemed capable of conveying the infection from the fields to human settlements

Brown,[7] like Jellison,[88] drew special attention to a North American species, the burrowing owl (*Speotyto cunicularia*) which lived in close contact with ground squirrels, often sharing their burrows The dangerous role of this owl in the spread of plague was confirmed by Wheeler & Douglas [211] through inoculation tests with pools of sticktight fleas (*Echidnophaga gallinacea*), common ectoparasites of the ground squirrels which had been collected from one such bird

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Chapter 7

INSECT VECTORS

FLEAS

Classification

While it is generally agreed that the fleas belong to the class Insecta of the phylum Arthropoda different names have been used to designate the order into which these insects fall British and American workers preferring the term Siphonaptera continental European authors that of Aphaniptera

The known genera of fleas are placed in two superfamilies—namely Pulicoidea and Ceratophylloidea The family of Pulicidae which belongs to the former superfamily is of greatest importance as far as plague is concerned because it comprises notorious vectors like certain Xeno psyllinae Descriptions of the outstanding characteristics of these and other species of interest to plague workers will be found in Annex 2 (see page 648)

Development

There are four stages in the life cycle of a flea the larvae hatched from the eggs passing through a pupal stage before becoming adults The early stages of this life cycle may be described as follows

The eggs minute glistening and white in colour are deposited either on the hosts or in their nests or haunts Sometimes eggs laid on the hosts stick to the pelt or plumage of the latter but even then they usually fall off sooner or later

The number of eggs laid by the female flea apparently differs in different species As stated by Patton & Evans[197] a female *Xenopsylla cheopis* lays 300-400 eggs during her lifetime—2 to 6 or even more at a time Bacot[6] observed a female *Pulex irritans* depositing 448 eggs during a period of 196 days while according to Patton[196] the female cat flea (*Cteno*

cephalides felis) lays over 800 eggs throughout her life span Oviposition occurs only when the female has regular blood meals

As described by Jordan,[1 1] 'the larvae that hatch from the eggs are rather like small legless caterpillars, they are pallid or slightly brown and bear sparse long hairs on all segments of the body When disturbed they throw themselves about with a twisting motion are very lively and dislike light they sometimes occur on the hinder part of mice in the pelts of dirty dogs and cats, on nestling birds and occasionally on human beings of unclean habits , but usually live on floors or in the nests or lairs of the hosts where they are able to feed on organic matter contained in the dust

Dealing with the next stage in the development of the fleas Jordan[131] stated that

"when ready to pupate the larvae make semi transparent cocoons by sticking bits of earth sand or organic refuse together amongst the debris or they may fill small cavities in hollow trees etc with a loose network of silken threads so as to support the cocoons of numerous individuals

The larvae remain quiescent within the cocoons for a time and then change to pupae from which the adults eventually emerge, the adults may remain fully formed within the pupal skin for some time before emerging Since the walls of the cocoon are opaque it is usually impossible to tell whether it contains a larva, a pupa or an adult flea and the period passed within the cocoon is therefore termed, for convenience, the cocoon stage'

While, under optimal conditions, the life cycle of a flea may be completed within three weeks, unfavourable climatic influences or conditions of starvation may considerably prolong the various stages of development Laboratory observations on the maximal duration in days of this development, and also on the length of adult life in four common flea species were summarized by Bacot[6] as follows

Species	*Egg stage*	*Active larval stage*	*Cocoon stage*	*Length of active adult life* fed	unfed	*Total days (fed adults)*
Nosopsyllus fasciatus	10	114	450	106	95	680
Xenopsylla cheopis	10	84	182	100	38	376
Pulex irritans	12	202	239	513	125	966
Ctenocephalides canis	8	142	354	234	58	738

Length of adult life

It has often been maintained that unfed fleas live longer than those which have partaken of single blood meals because a flea which has fed once must continue to do so in order to survive However, as noted below, the experiences of some observers did not bear out this contention

General agreement exists that female fleas survive longer than the males of the corresponding species In the opinion of some observers the lower ratio of surface area to mass in the females renders them less sensitive to untoward climatic conditions particularly low humidity

Since fleas may find more favourable conditions for survival on their specific hosts and still more in inhabited burrows or other sheltered locations than under artificial conditions it is difficult to deduce from laboratory observations the normal length of life of these insects Commenting on the findings tabulated earlier Bacot [6] maintained that

> on this basis and allowing for the longest recorded *unfed* imaginal lives it will be seen that there is no difficulty in accounting for active adults being found in favourable situations where there have been no hosts for considerable periods We may safely estimate for *C* [N] *fasciatus* 22 months *P irritans* 19 months *X cheopis* 10 months *Ct canis* 18 months "

Observations made in the case of the species infesting the sisels (susliks) of south east Russia have shown that wild rodent fleas may also survive for prolonged periods Golov & Ioff [8 83] maintained in this connexion that suslik fleas could stand low temperatures—down to −25°C (−13°F)—and prolonged starvation up to 10 months Fleas kept in test tubes at temperatures corresponding to those of the rodent burrows in winter remained active and able to feed upon hibernating susliks Large scale investigations carried out later by Tiflov & Ioff [2 5] showed that

(1) Fleas of the south east Russian wild rodents kept starving in a moist atmosphere could survive for prolonged periods—at room temperature up to 292 days in the cellar up to 369 days The fleas inhabiting the nests instead of dwelling upon the hosts themselves appeared especially well adapted to prolonged starvation

(2) Fleas fed once soon after leaving their cocoons generally survived a little longer than those of the preceding series

(3) Those fed periodically survived up to a maximum of 1 725 days i e *4 years 8 months and 25 days in the case of a female Neopsylla setosa*

(4) Under unfavourable conditions of humidity the female fleas proved hardier than the males

Further studies on the longevity of adult fleas were made by Burroughs [6] with over 10 000 specimens of different fleas which were kept in the laboratory at various temperatures and humidities within ranges encountered under natural conditions The maximal periods of survival of six species infesting wild rodents in the USA were found to compare with the length of life of X *cheopis* and *N fasciatus* thus

Species	Maximal length of life in days by sexes		
	Unfed	Fed once	Fed daily
Diamanus montanus	45 ♀	52 ♀	331 ♀
Oropsylla idahoensis	37 ♀	43 ♀	352 ♀
Malaraeus telchinum	15 ♂	20 ♀	182 ♀
Opisodasys nesiotus	15 ♂	17 ♀	48 ♂
Orchopeas sexdentatus	14 ♂	15 ♀	319 ♀
Megabothris abantis	10 ♂	19 ♀	477 ♀
Xenopsylla cheopis	40 ♂	41 ♂	158 ♀
Nosopsyllus fasciatus	13 ♂	22 ♂	281 ♀

Burroughs concluded from his observations that

(a) generally speaking, a single blood meal proved beneficial for the fleas, those fed once before starvation surviving a little longer than those never fed

(b) the longest lived fleas were those kept at high humidity (relative humidity of 90 % or more) and fed daily

(c) with one exception (*Opisodasys nesiotus*, which could not be kept satisfactorily), the maximum periods of longevity were among the females

Influence of climatic conditions

Many observations have shown that a close relation exists between the prevailing climatic conditions and the incidence of the various flea species If the climatic conditions are favourable for the development and survival of a given species, its incidence will be high Conversely, deviations from such favourable weather conditions are bound to lead to a decreased occurrence of the fleas in question It is clear, therefore, that periodic changes of the climatic conditions are apt to be followed by seasonal fluctuations in the incidence of the fleas and that since their climatic requirements vary considerably, different species of fleas may be predominant at different seasons of the year Consequently it is often not so much the total frequency of the fleas as the relative frequency of the different flea species which is at variance in a given locality, or on a given host, during the different seasons

The problem of how climatic factors influence the flea incidence has been the subject of many investigations Some observers e g Nicoll,[185] Bacot & Martin [9] and Goyle,[87] came to the conclusion that a high saturation deficiency (drying power of the atmosphere [9]) exerted an unfavourable influence on the adult fleas Bacot and Martin [9] stated in this connexion that

1 The survival of fleas (*X cheopis*) apart from their host is approximately in inverse proportion to the saturation deficiency of the air provided the temperature and air movement are constant In other words it is proportional to the rate at which they lose water

2 Under similar conditions but with constant saturation deficiency their length of life is reduced to between one half and two thirds by [a] 10°C [18 F] rise in temperature Compared with the effect of saturation deficiency that of temperature upon the longevity of fleas is within the range of climatic conditions over the greater part of India a smaller one

3 A variation in saturation deficiency from 5 mm to 35 mm [0 2 1 4 inches] such as occurs in the plains of Northern India at different seasons would accordingly shorten the average duration of life of wandering rat fleas in the proportion of 15 to 1 As a rise in mean temperature occurs simultaneously with the increase in saturation deficiency and may amount to a difference of 20°C [36°F] between January and June this would reduce the length of life of wandering fleas to about one third The effect of saturation deficiency and increased temperature will be additive and would go a long way to explain some of the climatological features of the epidemics "

The validity of the above statements was denied by later observers first apparently by Leeson[146] who found that there was no direct proportion between survival of unfed fleas and saturation deficiency of the atmosphere at any temperature He again came to the same conclusion when working with fleas which had been fed before being subjected to starvation[14]

Mellanby[1 0] found that while adult fleas were comparatively resistant to dryness of the atmosphere the larvae possessed no such resistance His results were corroborated by Buxton[9] who pointed out that owing to the presence of regulating mechanisms the loss of water by adult fleas was insignificant even at high temperatures and low humidities and could be made good at the next meal The flea larva on the contrary was not capable of much resistance to desiccation losing water with its excrement and probably also in respiration As established by Sharif,[2 9] an adequate moisture content of the food which formed the chief source of water supply for the flea larvae was essential for their development However an excessively high humidity because it rendered the food unsuitable was as inimical for the development of the larvae as too low a humidity[30]

It is of great importance to realize that even if the extrinsic climatic conditions become unfavourable the micro climate prevailing in the rodent burrows may continue to be suitable for the development of the fleas[9] Ingram[1 5] noted in this connexion that the temperature in the rodent burrows was higher during the winter and lower during the summer than the outside temperature whereas the humidity in the burrows was constantly higher than that outside A series of observations made by George & Webster[71] showed the following

	Maximum	*Minimum*	*Daily range*
Outside temperature	86 5 F (30 3 C)	63 F (17 2 C)	12 5°F to 21°F (7°C to 13 8°C)
Temperature in burrows	79 F (26 1 C)	72°F (22 2°C)	1 F to 7°F (0 55 C to 3 8 C)

Thus as summarized by these workers the temperature in the burrows was affected only to a small extent by the daily variations of the outside temperature

Comparisons of the state of humidity showed that the saturation deficiency of the air in the burrows varied from 0 11 inch to 0 14 inch (2 8 mm to 3 6 mm) whereas that of the outside air varied from 0 65 inch to 0 19 inch (16 5 mm to 4 8 mm)

The conclusions drawn from further observations recorded in India in 1936[117] were that the temperature of rat burrows almost corresponded to the minimum temperature of the outside air and exhibited only a limited range of diurnal variation and that the saturation deficiency was decidedly lower than that of the outside air

Macchiavello[156] laid great stress upon the fact that in some areas of Brazil where the weather was exceedingly hot, *Y cheopis* found suitable conditions for survival in the rat burrows where the temperature was 5° 8°C (9°-14°F) lower than outside

Interesting observations were also recorded by Sharif & Narasimham[231] in connexion with differences in flea infestation detected by them in the case of three wild rodent species Ascribing these differences in the flea index of the animals to the different arrangement and depth of their burrows the two workers stated that

> *M* [*Millardia*] *meltada* usually lives in a straight burrow which hardly goes more than 9 inches [23 cm] deep into the soil and is open at both ends As the burrow is not deep the direct heat of the Sun raises the temperature of the nest and keeps it hot and dry This makes it impossible for any flea to breed in the nest of this rodent The burrows of *T* [*Tatera*] *indica* go as deep as 40 inches [1 m] and they have a tortuous course The heat of the Sun cannot penetrate this depth and ventilation is very poor on account of the tortuous course of the burrow The temperature in the nest can only be lower and humidity higher than those of the atmosphere The temperature and humidity of such deep burrows are least affected by atmospheric changes They remain almost constant with slight seasonal variations at such a depth The nest of *T indica* is thus an ideal place for breeding of fleas and consequently it is always full of fleas and as the result of that this species always harbours the largest number of fleas The burrow of *G* [*Gunomys*] *kok* is intermediate between the two burrows The nest is found at a depth of 12 to 18 inches [30-45 cm] but the course is such as to encourage ventilation It is not so favourabl for breeding of fleas as that of *T indica* Consequently very few fleas are found in it On the whole there was [a] slight diminution in the flea infestation of *T indica* during the hot months but it was observed that the flea infestation was directly proportional to the depth of a burrow during these months

Estrade[56] pointed out the important fact that *X cheopis* could thrive in accumulations of dust containing cereal debris, where the temperature was apt to be constant, little ventilation took place, and the saturation deficiency varied little As a result, young fleas could develop by the hundred in dark corners of the huts in Madagascar[4, 8, 81, 217] In the opinion of Girard,[76] such ' free living fleas were apt to obtain their first meal from man rather than from rats and if feeding on a patient with plague bacteraemia, might become a means of conveying the infection to the rats

Observations by Herivaux & Toumanoff[93] had shown that, in Saigon, Indochina *X cheopis* was mainly found in houses, the floors of which were paved with tiles made from baked clay, or in huts with a soil of sandy clay whereas *X astia* was prevalent in huts with a sandy soil Attempting to explain the reason for these differences in the distribution of the two flea species, Toumanoff & Herivaux [59] suggested that the evaporation of water accumulated in the sandy clay or from the baked clay tiles led to a lowering of the temperature and thus created favourable conditions for *X cheopis* which preferred, in comparison with *X astia* a lower temperature

Observations made in India[119] showed that the maximum temperature in cracks of the walls of houses and in thick thatch roofs was 5° 10° F (3° 6°C) below that of the outside air Possibly, therefore such locations are also suitable for the development of fleas

It should be noted in this connexion that the humidity requirements for the development of different flea species may vary considerably Thus de Meillon[169] found that X brasiliensis because it was able to complete its life-cycle at a relative humidity of 51 % could breed in debris accumulated on the floor of huts sheds or garages even though the humidity there was much lower than that prevailing in rodent burrows

Ecology

Although dependent upon their hosts for nourishment the adult fleas of many species do not stay on them constantly but spend a considerable part of their lives in the nests of the rodent burrows or even lead a rather independent existence as free living fleas

It is important to note that depending on the species concerned and on the prevailing climatic conditions the proportion of time the fleas spend on the hosts and away from them respectively, is subject to much variation

As far as the common rat fleas are concerned it holds generally true that *N fasciatus* which feeds at long intervals spends most of its time off the body of its hosts whereas *X cheopis* which feeds frequently has a far greater tendency to stay in the fur of the rats[98]

Making comparative observations on *X cheopis X astia* and *X brasiliensis* Webster & Chitre [7] stated that

" a very large proportion of all the fleas was found on the host It is not impossible that the wandering may be largely nocturnal but the figures suggest that the three species show no marked difference in the proportions on hosts and abroad respectively

The probability that the fleas which shun daylight move around mainly after dark deserves attention Some observers e g Sanguy [1] maintained that the fleas convey plague to man mainly at night

Though the rat *Xenopsylla* no doubt have a tendency to infest their hosts more permanently than *N fasciatus* it is important to keep in mind that as has been noted earlier they have been found able to lead under suitable environmental conditions a rather independent existence as free living fleas This observation has been made in India as well as elsewhere[137]

Reporting on their observations in the Lake Albert plague focus of the Belgian Congo Vincke & Devignat [65] stated that in contrast to *X cheopis* and *X brasiliensis* which were apt to lead an independent existence on cereal debris accumulated in dark and unswept corners of the straw huts *Leptopsylla segnis* was found exclusively on the hosts These observations on *L segnis* which is a blind flea stand in an interesting contrast to those of Tiflov & Potapov [56] in south east Russia these workers maintained that flea species with well developed eyes such as *Citellophilus tesquorum* and

Frontopsylla semura, had a tendency to stay on their wild rodent hosts while fleas with rudimentary eyes (*N setosa* and *Ctenophthalmus pollex*) inhabited the nests However, no stringent conclusions should be drawn because, in Madagascar, *L segnis* was found in the burrows as well as on the rodents [217]

Little doubt can exist that the proportion of fleas found on their hosts, and away from them, is influenced by the prevailing climatic conditions Indeed, it seems likely that, in this respect, the basic differences found between the *Xenopsylla* living in warm countries like India and *N fasciatus* which is the prevalent rat flea in countries with a cooler climate, are not wholly due to intrinsic causes, but are partly the result of climatic influences

That climatic conditions are apt to exert an influence upon the frequency of fleas on their hosts has been proved by observations like that of Hirst,[10] who found that rainy weather led to temporarily lowered flea counts

Impressed by the role which fleas leaving the carcasses of plague rats played in the spread of the infection, early workers were led to believe that this exodus took place rather rapidly In this connexion, Webster & Chitre[5] quoted a statement by Mason [161] to the effect that the fleas began to leave a carcass 15 seconds after a rat had died and that all had departed after two hours and a quarter However, Webster & Chitre pointed out that in their experience, even when live rats were close at hand, active fleas could be regularly recovered from the carcasses after the latter had begun to putrefy Similar observations were recorded by other workers for instance, by Martinez,[163] who stated that he had found more than 30 fleas on one dead rat Roberts, [16] while agreeing that statements relating to all fleas leaving their late hosts immediately or very soon after death are not accurate , emphasized that certain species, particularly the rat *Xenopsylla* left their hosts at greater speeds than others, e g the field rodent fleas *Dinopsyllus ellobius lypusus* and *Ctenophthalmus calceus*

Cole & Koepke,[35] analysing the results of a rat flea survey at Savannah Ga computed that the rats delivered dead to the laboratory had lost 70 4% of their *X cheopis*, but only 56 7% of their *L segnis* These authors added, however, that this difference was not statistically significant

In the course of his work in China, Pollitzer (unpublished observations) gained the distinct impression that the number of fleas on rats delivered dead to the laboratory was larger when the weather was temporarily inclement It is noteworthy in this connexion that, according to Robic [217] a lower temperature exerts an adverse influence on the activity of the fleas

As proved by the observations of Symes & Hopkins [19] fleas of the species *Echidnophaga gallinacea* usually remain attached to the dead rodents

Nutritional requirements

Larvae Marked differences exist in the nutritional requirements of the larvae of different flea species Some the *N fasciatus* larvae for instance

depend upon the presence of blood substances in their food which under natural conditions are supplied to them in the faeces of the adult fleas For other flea larvae e g those of the *Xenopsylla* such substances are not indispensable although favourable for their development

It will be perceived that in contrast to fleas like the *Xenopsylla* the propensity of adult *N fasciatus* to dwell in the nests rather than on the bodies of their hosts is a factor of great importance in the survival of this species

Profound studies on the nutritional requirements of the larvae of *N fasciatus* and the Indian rat fleas (*X cheopis X astia* and *X brasiliensis*) by Shariff [2 8] showed that

(1) Though blood was essential for the larvae of *N fasciatus* it was not fully sufficient for larval development the larvae requiring additional food substances which in nature were supplied to them by the organic refuse present in the bed of the host of the adult fleas [7]

(2) Pure blood also proved inadequate for the larval development of the *Xenopsylla* because it was deficient in accessory growth factors particularly vitamins of the B group

(3) In nature the larvae of these fleas apparently derived the accessory food substances from micro organisms possibly fungi admixed to their food It was obviously due to the presence of such micro organisms that the *Xenopsylla* larvae could subsist on wheat flour

Confirming the statements of earlier observers e g Hirst [98] Sharif reached the conclusion that the distribution of the various rat flea species was governed by the character of the food supply available to their larvae as well as by climatic factors Particularly important was that in contrast to *X astia* which needed comparatively the most nutritive diet the larvae of *X cheopis* and *X brasiliensis* had simple nutritional requirements Consequently they were apt to survive transport in grain even without rats to places far from their original home [8]

Nevertheless since the temperature tolerance of *X brasiliensis* was low its incidence in India was confined to cooler regions *X cheopis* being more adaptable in this respect was on the contrary widely distributed

Adults Though adult fleas of both sexes are normally dependent for subsistence on the blood which they suck directly from their hosts it is curious to note that according to Jordan [131] a starved flea will imbibe blood oozing from a pin prick will suck at a drop of water or even insert its mouth parts into the skin of a caterpillar and suck its body fluids

The frequency with which adult fleas obtain meals from their hosts seems to depend not only on the species concerned but also on the sex of the fleas and on climatic conditions

Frontopsylla semura, had a tendency to stay on their wild rodent hosts, while fleas with rudimentary eyes (*N setosa* and *Ctenophthalmus pollex*) inhabited the nests However, no stringent conclusions should be drawn because, in Madagascar, *L segnis* was found in the burrows as well as on the rodents -[17]

Little doubt can exist that the proportion of fleas found on their hosts and away from them, is influenced by the prevailing climatic conditions indeed it seems likely that in this respect, the basic differences found between the *Xenopsylla*, living in warm countries like India, and *N fasciatus*, which is the prevalent rat flea in countries with a cooler climate, are not wholly due to intrinsic causes, but are partly the result of climatic influences

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Nutritional requirements

Larvae Marked differences exist in the nutritional requirements of the larvae of different flea species Some the *N fasciatus* larvae for instance

With regard to the differences existing in this respect betwee flea species, it has already been noted that among the common N *fasciatus* feeds at considerably longer intervals than the *Xenops*

That there may be a difference, in respect of the frequency of between the sexes of the same flea species has been shown in the *X cheopis X astia*, and *X brasiliensis* the males of which ha found to attack their hosts more frequently than the females [97]

Discussing the influence exerted by climatic factors on the fre with which the fleas bite, Hirst [98] considered it likely that a high satu deficiency, by rendering the fleas more thirsty, would induce them t more often That a high temperature may act in an analogous m has been shown by the investigations of Cole [31] who maintained how that temperature variations exerted an influence only on *X cheopis* m which fed more frequently in hot weather and less frequently if the temp ture was lower

Host selectivity

Though most rodent fleas specifically infest more than one specie of animal, as a rule they restrict themselves to hosts belonging to the same generic group [51] Though they may be reluctant to do so they are neverthe less capable of attacking hosts belonging to other generic groups, if driven by hunger Therefore as aptly pointed out by Wayson, the host pre ferences of the rodent fleas merely retard but do not prevent their transi tion from one genus of hosts to another

Of particularly ominous importance is the fact that with the exception of *L segnis* the fleas commonly infesting the commensal rodents quite readily attack man if thoroughly starved The rat *Xenopsylla* in particular have been found able to subsist for considerable periods on human blood alone as shown by the following data supplied by Webster & Chitre [274]

Rat fleas fed on human blood only

Species	*Length of l fe (days)*	*Feeds*		*Pe centage accept d*
		offered	*accept d*	
X cheopis female	162	136	42	30 88
Y cheopis male	63	53	28	52 83
X astia female *	53	47	13	27 66
X brasiliensis female	127	57	31	54 38
X brasiliensis male	68	60	29	48 33

* No male *astia* was kept alive on human blood for more than a few days although many fed readily

Webster [73] compared the percentages of food acceptance by the three pecies of Indian rat fleas when exposed daily on rats guinea pigs and a man subject, respectively, and recorded the following results

Species	*Percentages of acceptance when fed on* rats	guinea pigs	men
X *cheopis* female	51	53	32
X *cheopis* male	67	75	46
X *astia* female	64	55	38
X *astia* male	84	91	52
X *brasiliensis* female	47	-	42
X *brasiliensis* male	72	—	44

It will be noted that, even when fed on rats the fleas did not daily feeds No marked differences existed between the three flea particularly as far as the tests made on the human subject were cor Webster s conclusion was therefore that the three Indian roden *psylla* readily feed on man in the absence of a more suitable host temperatures of over 80°F [27°C]

Though as shown in the lists of wild rodent fleas given in A table V (see page 638) a considerable number of the species have bee capable of biting man it has been often maintained particularly by in the western States of the USA [51 5 1 3 1 4] that these fleas attack i more reluctantly than the rat fleas and are therefore by no means in responsible for the transmission of plague from the wild rodents t Apparently however differences exist in this respect between d flea species Thus Meyer & Holdenried [1 8] referring to ecological instituted to trace the origin of human plague infection in rural a California stated that fleas of the Muridae—mice (*Peromyscus*) n mice (*Microtus*) and pack rats (*Neotoma*)—in the vicinity of human tions were the most likely transmitters of *Pasteurella pestis*

Natural enemies

Observations by several authors have shown that fleas are preye by certain other insect species In this respect an important rôle is by the ants which are able to attack not only flea eggs and larva also adult fleas [3] The latter are also destroyed by spiders [73] whil insect species attack the eggs larvae or pupae

Fedina [60] and Flegontova [6] found that certain beetles part those of the Staphilinidae family preyed upon the larvae and ac fleas in the burrows of *Citellus pygmaeus* Flegontova expres opinion that the activity of the beetles reduced the likelihood survival of the fleas in the wild rodent burrows and consequent chances for a carry over of plague by the fleas

Commensal Rodent Fleas

Classification [a]

The fleas found on the commensal rodents of the various plagu may be classed as follows

[a] Th m mporta t ta o omi ch racters of fle as w ll a key to the d nt f atk of som fl are co t ed i A x (p g 648)

(1) Those specific to the commensal rodents which show a wide distribution or are found in several plague areas X *cheopis* falls into the first category, *X brasiliensis* and *N fasciatus* into the second *L segnis*, though considered by most authorities to be specific to the commensal mice, should also be included in the second group because it is regularly found on the commensal rats in many areas, usually the numbers found are small, but occasionally the percentage is higher

(2) Those specific to the commensal rats which show a more limited or even a quite restricted geographical distribution, such as *X astia* and some other fleas which are mentioned later

(3) Fleas of wild rodents which *may infect the commensal species* either accidentally or, even, with some regularity

(4) Flea species which, because they are frequent in the environment of the commensal rodents, are often found in limited numbers on the latter *although they are not specific for them Echidnophaga gallinacea* and *P irritans* two species with an almost cosmopolitan distribution and a remarkable faculty for adapting themselves to a large range of hosts, stand foremost in this group but some other species, particularly *Ct felis*, are also included

Factors governing incidence

It has been claimed by some authors that *X cheopis* is a parasite of *Rattus rattus* rather than of *Rattus norvegicus*, but no doubt can exist that the observations which seemed to prove this contention are not generally valid As a rule, in localities where *R norvegicus* and subspecies of *rattus* coexist, *X cheopis* infests both indiscriminately and often, even *predominates to some extent, on R norvegicus Occasionally, an unusually* high *cheopis* infestation of *R norvegicus* has been observed Eskey [57] collected at San Francisco, Calif, 1,600 X *cheopis* from 10 Norway rats trapped within a period of 10 days in the basement of one building

X cheopis has also been found to be the main rat parasite in localities where Norway rats greatly preponderated—for instance in Manchuria [110, 191] Likewise Hecht [91] found a 98% incidence of *R norvegicus* and a 95% *cheopis* incidence at Caracas Venezuela

It seems safe therefore, to state that *X cheopis* shows no specific preference for the *rattus* subspecies, but parasitizes the Norway rats at least to the same degree As a rule, this flea is considerably less common on commensal mice and shrews

According to observations made in India, other rodent species with opportunities for contact with the commensal rats showed, as a rule slight infestation with *X cheopis* but were apt to be more highly infested with *X astia* than the two rat species [71, 3, 75] As stated by Hirst [101] the latter flea was also widespread on insectivores

Incidence in relation to climatic conditions

The statement made earlier that different flea species react differently to the influence of climatic conditions is well supported by many observa tions made in respect of the rat fleas

As far as the rat *Xenopsylla* are concerned it may be stated that *X astia* possesses a poor power of adaptation to temperate climatic conditions being particularly sensitive to low temperatures[96, 101, 3]

According to observations in India[1, 3, 273] *X brasiliensis* was on the contrary adversely affected by hot weather whereas the coolest season was congenial for it It is in accord with these findings that *X brasiliensis* is prevalent in the temperate section of Brazil[11, 15] However Roberts[211] pointed out that *in Kenya this flea was able to thrive in the hot humid* coastal areas as well as on the cold damp mountain slopes and also in very dry areas with a high temperature Nevertheless *X brasiliensis* showed a limited geographical distribution in Kenya a fact partly explained in Roberts opinion by its failure to colonize in the underground burrows of *R rattus*

As shown by its wide geographical distribution *X cheopis* is able to adapt itself to a considerable range of climatic conditions It is as has been previously noted the common rat flea in Manchuria and is also the prevalent species in the tropical section of Brazil[14, 15]

Comparing the distribution of *X cheopis* with that of *X astia* Hirst[104] drew attention to the observations of King & Pandit[137] who came to the conclusion that the prolonged duration rather than the intensity of the hot weather accounted for the scarcity of *X cheopis* in the south east of Madras Presidency (now Madras State) Hirst maintained however that

" *the intense hot and dry weather of the Ganges plains exerts no more than a temporary* check on the great cool weather abundance of this flea in most parts of that vast plague stricken region Undoubtedly climate has an important influence on the distribution of these two fleas but the salient point seems to be the sensitiveness of *astia* to cold not that of *cheopis* to heat

Nevertheless there can be no doubt that generally speaking a moder ately warm and moist climate is optimal for *X cheopis* Thus Estrade[56] established that on the Madagascar plateau the optimum conditions for this flea were a temperature of 15° 20°C (59° 68°F) and a relative humidity of 85% to 95% Mohr[18] dealing with the situation in the USA found that the *cheopis* populations were restricted to warm humid zones becoming increasingly sparse *in the cold arid areas The incidence of X cheopis* was generally highest in localities where the January mean temperature was 40°F (4 5°C) or more

It is in accordance with these climatic predilections that if established in a tropical climate *X cheopis* prefers cooler locations as a rule[131] Park[195]

(1) Those specific to the commensal rodents which show a wide distribution or are found in several plague areas. *X. cheopis* falls into the first category, *X. brasiliensis* and *N. fasciatus* into the second. *L. segnis* though considered by most authorities to be specific to the commensal mice, should also be included in the second group because it is regularly found on the commensal rats in many areas, usually the numbers found are small, but occasionally the percentage is higher.

(2) Those specific to the commensal rats which show a more limited or even a quite restricted, geographical distribution, such as *X. astia* and some other fleas which are mentioned later.

(3) Fleas of wild rodents which may infect the commensal species either accidentally or even, with some regularity.

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It has been claimed by some authors that *X. cheopis* is a parasite of *Rattus rattus* rather than of *Rattus norvegicus*, but no doubt can exist that the observations which seemed to prove this contention are not generally valid. As a rule, in localities where *R. norvegicus* and subspecies of *rattus* coexist, *X. cheopis* infests both indiscriminately and often, even predominates, to some extent, on *R. norvegicus*. Occasionally, an unusually high *cheopis* infestation of *R. norvegicus* has been observed. Eskey[5] collected at San Francisco, Calif., 1,600 *X. cheopis* from 10 Norway rats trapped within a period of 10 days in the basement of one building.

X. cheopis has also been found to be the main rat parasite in localities where Norway rats greatly preponderated—for instance, in Manchuria.[110, 191] Likewise Hecht[91] found a 98% incidence of *R. norvegicus* and a 95% *cheopis* incidence at Caracas, Venezuela.

It seems safe, therefore, to state that *X. cheopis* shows no specific preference for the *rattus* subspecies, but parasitizes the Norway rats at least to the same degree. As a rule, this flea is considerably less common on commensal mice and shrews.

According to observations made in India, other rodent species with opportunities for contact with the commensal rats showed, as a rule, slight infestation with *X. cheopis* but were apt to be more highly infested with *X. astia* than the two rat species.[1, 23, 25] As stated by Hirst[101] the latter flea was also widespread on insectivores.

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"... the intense hot and dry weather of the Ganges plains exerts no more than a temporary check on the great cool weather abundance of this flea in most parts of that vast plague-stricken region. Undoubtedly climate has an important influence on the distribution of these two fleas, but the salient point seems to be the sensitiveness of *astia* to cold, not that of *cheopis* to heat."

Nevertheless, there can be no doubt that, generally speaking, a moderately warm and moist climate is optimal for *X. cheopis*. Thus Estrade [56] established that on the Madagascar plateau the optimum conditions for this flea were a temperature of 15°-20°C (59°-68°F) and a relative humidity of 85% to 95%. Mohr [18], dealing with the situation in the USA, found that the *cheopis* populations were restricted to warm, humid zones, becoming increasingly sparse in the cold, arid areas. The incidence of *X. cheopis* was generally highest in localities where the January mean temperature was 40°F (4.5°C) or more.

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According to observations made in India other rodent species with opportunities for contact with the commensal rats showed as a rule slight infestation with *X cheopis* but were apt to be more highly infested with *X astia* than the two rat species[71, 3, 75] As stated by Hirst[104] the latter flea was also widespread on insectivores

Incidence in relation to climatic conditions

The statement made earlier that different flea species react differently to the influence of climatic conditions is well supported by many observations made in respect of the rat fleas.

As far as the rat *Xenopsylla* are concerned, it may be stated that *X. astia* possesses a poor power of adaptation to temperate climatic conditions being particularly sensitive to low temperatures.[98, 101, 3]

According to observations in India[1, 3, 2, 3] *X. brasiliensis* was, on the contrary, adversely affected by hot weather, whereas the coolest season was congenial for it. It is in accord with these findings that *X. brasiliensis* is prevalent in the temperate section of Brazil.[14, 15] However, Roberts[14] pointed out that in Kenya this flea was able to thrive in the hot, humid coastal areas as well as on the cold, damp mountain slopes and also in very dry areas with a high temperature. Nevertheless *X. brasiliensis* showed a limited geographical distribution in Kenya, a fact partly explained in Roberts' opinion by its failure to colonize in the underground burrows of *R. rattus*.

As shown by its wide geographical distribution, *X. cheopis* is able to adapt itself to a considerable range of climatic conditions. It is, as has been previously noted, the common rat flea in Manchuria and is also the prevalent species in the tropical section of Brazil.[14, 15]

Comparing the distribution of *X. cheopis* with that of *X. astia*, Hirst[101] drew attention to the observations of King & Pandit,[137] who came to the conclusion that the prolonged duration rather than the intensity of the hot weather accounted for the scarcity of *X. cheopis* in the south-east of Madras Presidency (now Madras State). Hirst maintained, however, that

> "... the intense hot and dry weather of the Ganges plains exerts no more than a temporary check on the great cool weather abundance of this flea in most parts of that vast plague-stricken region. Undoubtedly climate has an important influence on the distribution of these two fleas, but the salient point seems to be the sensitiveness of *astia* to cold, not that of *cheopis* to heat."

Nevertheless, there can be no doubt that, generally speaking, a moderately warm and moist climate is optimal for *X. cheopis*. Thus Estrade[56] established that on the Madagascar plateau the optimum conditions for this flea were a temperature of 15°-20°C (59°-68°F) and a relative humidity of 85% to 95%. Mohr,[18] dealing with the situation in the USA, found that the *cheopis* populations were restricted to warm, humid zones, becoming increasingly sparse in the cold, arid areas. The incidence of *X. cheopis* was generally highest in localities where the January mean temperature was 40°F (4.5°C) or more.

It is in accordance with these climatic predilections that, if established in a tropical climate, *X. cheopis* prefers cooler locations as a rule.[131] Park[195]

discussing the situation in India and Ceylon, aptly pointed out in this connexion that this flea is so hardy that it can thrive in the warm coastal strips, but in such cases prefers the commercial areas, where certain artificial conditions associated with commerce possibly compensate for more favour able climatic conditions naturally found on the hills

A point of great interest is that at the highest elevations of Ceylon (5,000 8,000 feet (1,524 2,438 m)), *X cheopis* is replaced by two indigenous rat fleas *Ceratophyllus tamilanus* and *Stivalius phoberus*[104] Similarly it was found by Macchiavello[16] that, in the highlands of the Andes above 3,000 m (about 9,850 feet) *Nosopsyllus londinensis* took the place of *X cheopis*

In view of their palaeo arctic origin, it is not surprising to find that *N fasciatus* and *L segnis* are both apt to be more abundant during the cold seasons than in summer[35 40] Hirst[104] found that the latter species together with *X cheopis* replaced *X astia* in the highlands of Ceylon at 4,000 5 000 feet (1,219 1,524 m)

As shown by the findings of Mohr,[18] in the USA *N fasciatus* was adversely affected by an arid or an extremely cold climate

Monopsyllus anisus which largely replaces *N fasciatus* in temperate, eastern Asia was, according to observations made in Shanghai prevalent in spring[91 280]

Incidence in relation to environmental conditions

In presenting the problem now under review, it has seemed advisable to deal separately with the influences exerted on the incidence of com mensal rodent fleas by climatic factors and by environmental conditions, respectively It must be realized, however that no sharp line of demarca tion can actually be drawn between these two factors, environmental conditions often exerting an indirect rather than a direct influence in this respect because they provide a microclimate favourable for the fleas

Workers in Ceylon, India, and Java are generally agreed that *X cheopis* has a marked predilection for comparatively dry situations as found for instance in granaries and warehouses Expressing this view, Hirst[10] added that observers in Java e g Otten, have stressed the point that it [*X cheopis*] is not adapted to multiply in nesting places out of doors or anywhere liable to marked excess of ground moisture In such situations the natural enemies of the larva (moulds mites and so forth) gain an ascendency

King & Pandit[137] found that insanitary surroundings seemed to favour *X cheopis* and suggested that this factor might be of importance in the spread of this species by compensating for the adverse influence of climatic conditions generally unsuitable for it

Studying the ecology of plague in two districts of Bombay State Sharif & Narasimham[3] found that, whereas *X cheopis* predominated in com

paratively dry and well ventilated houses, *X. brasiliensis* was prevalent in cool damp ill-ventilated houses. However, no general deductions should be drawn from the latter observation because, as mentioned earlier in this chapter, in South Africa and also in Kenya *X. brasiliensis* was found capable of thriving in excessively dry environments. Generally speaking, it would seem that local peculiarities of the environment are apt to cause marked differences in the incidence and distribution of the various rat flea species.

It is probable that changes in environmental conditions are apt to lead to changes in the incidence of rat fleas. This is suggested by an observation of Roberts[16] who found that *X. brasiliensis*, which was the predominant species in the initial stage of a plague outbreak in Kenya, steadily decreased in numbers during the outbreak, whereas *X. cheopis* increased and finally assumed a dominant position. Since it had been previously established that the latter flea mainly infested the rats living underground, Roberts was of the opinion that structural and other changes carried out in the houses during the outbreak created favourable conditions for an increase of *X. cheopis* because more rats were forced to live underground. *X. brasiliensis*, which mainly infested the rats living in the roofs, was at the same time adversely affected by these environmental changes.

Eskey, reporting in 1938 on a rat flea survey in San Francisco, Calif., noted an almost total absence of *P. irritans*, whereas 30 years earlier this species had averaged nearly one per rat trapped. It is quite likely that this decrease of *P. irritans*, which is an accidental parasite of the rats, was related to a gradual improvement in environmental sanitation.

It is of the greatest importance to state in conclusion that the incidence and species distribution of the commensal rodent fleas in any given locality are by no means invariably static but—even apart from seasonal fluctuations—are apt to be subject to marked changes caused, on the one hand, by an importation or infiltration of fleas and, on the other hand, by alterations in the local situation which influence the breeding and living conditions of the fleas either favourably or unfavourably.

It is clear that increased facilities for flea importation, combined with favourable local conditions, are apt to lead to the colonization of species hitherto not found or established in the locality in question.

For instance, such a situation favourable for *X. cheopis* seems to have developed in the port of Mattanchery in south-west India. As stated by Ali[3], a rat flea survey made there in 1930 had shown a low *brasiliensis* and *astia* incidence combined with an apparently total absence of *X. cheopis*. A second survey in 1937 established the presence of this species as well as an increased rat flea incidence in general. Ali ascribed these changes to a considerable increase in maritime traffic which had taken place during the period between the two surveys and which had obviously not been paralleled by provision of adequate storage facilities in the port.

Original homes and geographical distribution

Xenopsylla astia General agreement exists that *X astia* is an oriental flea Most observers consider India as its original home but Hirst [104] maintained it to be also indigenous in the lowlands of Ceylon, stating that ' it is very nearly the sole rat flea of the low wet zone outside Colombo

Apart from Ceylon and India, *X astia* is also established in adjacent countries such as Arabia Burma, Indochina Iran Iraq, and Java In Malaya, it was rarely found at Singapore by Gilmour [7] Though introduced *X astia* apparently became well established in a number of ports on the East African coast

According to a rat flea survey carried out in Bombay harbour in 1934 5 *X astia* was rarely found in ships, even though it was frequent in the dock area of that port [38] However this flea was abundant on barges surveyed in the river port of Rangoon [115] and it was found in small numbers on grain ships in Colombo harbour, particularly on those coming from Rangoon [104] Moreover there can be no doubt that the appearance of *X astia* on the east coast of Africa was due to its introduction by the rats of ships, probably Arabian dhows

Xenopsylla brasiliensis The original home of *X brasiliensis* the only member of its group which is found outside the Ethiopian region, was in all probability tropical Africa (Hopkins, personal communication) *X brasiliensis* is not only found in areas like the Belgian Congo Kenya and Uganda, which lie within or near, the zone of its original habitat it also occurs in the south of Africa

Since this flea frequently infests ship rats, it is not surprising to find that it became established in a number of areas overseas It was found in the Canary Islands [184] and, according to Jordan [131] also in England In Brazil it vies in importance with *X cheopis* being predominant in the temperate zone of that country, and according to Hecht [91] it was also found in Caracas, Venezuela In the East, it is a most important rat parasite in the central and southern uplands of peninsular India,[87] but is apparently rare in Ceylon [104]

Xenopsylla cheopis The question as to where the original home of *X cheopis* was situated has been the subject of considerable debate Hopkins, whom the present writer consulted in regard to this difficult problem stated that *X cheopis* is now so widely distributed partly as a result of natural spread and partly through the unintentional assistance of man that it seems impossible to decide whether it originated in the Nile Valley, as was suggested by Jordan & Rothschild [133] or in " Iran and Iraq and adjacent countries as suggested by Jordan [130] There is, of course no direct evidence because no flea specimens collected before the early nineteenth century are known to be in existence, but the balance of the indirect evidence possibly inclines a little in favour of a Nilotic origin for the species

The incidence of *X. cheopis* in the recently or currently plague-affected areas has been dealt with in chapter I. Generally speaking, though capable of adapting itself to a wide range of climatic conditions, it is most prevalent in warm countries. As emphasized by Jordan,[131] it occurs especially often outside its original range in ports and centres for the shipment of cereals. Widespread though *X. cheopis* is, it has even in recent times invaded new territories and apparently still continues to do so.

A point deserving attention in this connexion is the date when *X. cheopis* made its appearance in India. Elaborating a suggestion made by Hirst,[99] King & Pandit[1] suggested that

"... the explanation of the recent history of plague in India from 1896 onwards and why this is different from previously recorded epidemics which rapidly died out, is that whereas previous epidemics occurred in the absence of *cheopis*, the 1896 infection occurred when *cheopis* was fairly widespread throughout India as a whole.

"We suggest that the dissemination of *cheopis* occurred after the extension of human intercourse and trade (particularly the cotton trade) with Egypt following the opening of the Suez Canal in 1869. Probably the process of *cheopis* importation began even before this. Thus Choksy suggests that the actual infection was imported into Gujarat from Egypt in 1815 with cotton and caused the epidemic of that year. Whether this was so or not, it is apparent that the Bombay Presidency was importing Egyptian cotton at that time."

King & Pandit concluded, therefore, that

"... whereas all previous epidemics seem to have behaved like typical *astia* epidemics in being localized and in not carrying over from one year to another, the epidemic since 1896, as we in India know to our cost, has behaved very differently."

There can be little doubt that the ingenious hypothesis of King & Pandit deserves great attention as far as peninsular India is concerned. Whether it also holds true of northernmost India, particularly the plague areas in the foothills of the Himalayas, where the infection appears to have been more firmly entrenched, seems an open question. It would be most desirable to make a detailed review of the early history of the disease there, and in India in general, in the light of King & Pandit's hypothesis.

It has been claimed by some observers, e.g., Hirst,[10] Roubaud[18] and Girard,[80] that in past centuries *X. cheopis* was frequent in Europe and was thus apt to play the main role in the spread of the then frequent plague outbreaks there. It was suggested that the subsequent disappearance of *cheopis* from Europe might have resulted from the replacement of *R. rattus* by *R. norvegicus*, which is supposed to be a less suitable host for this flea.

Fascinating as this hypothesis is, it is difficult to accept it. Mention has already been made of the observations proving that *X. cheopis* has no specific predilection for *R. rattus*, being for instance abundant in Manchuria where Norway rats greatly predominate.

It might be argued that since *X. cheopis* had been able to establish itself in Manchuria, it could have done the same in Europe where the winters are considerably less cold. However, it is striking to note that

in Java formerly called *Pygiopsylla ahalae* has since been identified as *Stivalius cognatus*[140] Several *Stivalius* subspecies seem to be concerned in south India

2 *Synosternus pallidus* a flea occurring from Senegambia and Morocco to India and infesting a variety of hosts including rodents hedgehogs and carnivora (Jordan[131]) was found to be frequent in French West Africa mainly on the floors of the houses but also to some extent on the commensal rodents It is known to bite man[1, 136, 111]

3 *Synopsyllus fonquernei* a species occurring on the high plateau of Madagascar Originally identified on a small lemur and formerly apparently rare this flea has become since 1931 increasingly frequent on rats as well as on hedgehogs and insectivores (*tanrecs*) [9, 141, 1, 19] According to Girard[8] it is unknown whether it attacks man

4 *Paractenopsyllus kerguisteli* a flea closely allied to *L segnis* found on the Madagascar plateau on commensal rodents and dogs but probably also infesting many other species of mammals living in forests[19, 267]

5 *Xenopsylla vexabilis hawaiiensis* frequent on the commensal rodents in Hawaii where it probably plays a role as a plague vector in the fields[0, 89]

6 *Pulex irritans* an Old World flea now of almost cosmopolitan distribution Though often called (or one should rather say misnamed) the human flea it has actually a remarkably wide host range In addition to infesting man and usually in small numbers the commensal rats it often attacks pigs and goats as well as foxes and badgers from which according to Jordan[131] cave dwelling man probably derived the parasite

P irritans is the predominant flea of the domestic dog in China particularly in north China where *Ct canis* is almost totally absent It has also been found on dogs in other parts of the world e g in Brazil[63] England[1, 4] South Africa[163] and the western States of the USA[1, 6] In Manchuria *P irritans* was also common on the domestic cat[191]

This flea was also found on a wide range of wild animals including rodents hares and foxes in Argentina[13, 36, 20] foxes and wild cats in Alberta Canada[126] *Cavia aperea* and rabbits in Ecuador[49, 159, 163] domestic guinea pigs and *Sigmodon peruanus* in Peru[13] and rodents and carnivores in the western States of the USA[106, 111, 1, 6, 179]

7 *Ctenocephalides species* *Ct felis felis* and *Ct canis* of palaeo arctic origin have now become almost cosmopolitan occurring not only on their specific hosts—the cat and dog respectively—but also on other species of mammals often in small numbers on the commensal rats and occasionally on other rodents Both species of fleas are prone to attack man and not infrequently become serious pests in houses

Ct felis orientis considered by Jordan[131] as the specific flea of the dog in the Orient was also found on jackals and on rodents in India[31] and in

the colonies of *X cheopis* recently detected in Europe were almost invariably located in the basements of steam heated buildings where even in winter, the temperature was suitable for the development and survival of this warmth loving flea It seems rather doubtful whether similarly suitable temperatures were present during winter in the houses of mediaeval Europe It is of importance to note in this connexion that the frequency of *X cheopis* in Manchuria is probably due to the peculiar heating arrangements in many of the houses These are provided with wide, elevated platforms made from brick which, because they serve for sleeping as well as living purposes, are kept warm with the aid of flues day and night Identical conditions probably account for the frequency of *X cheopis* in Korea (Barnett & Tashioka [11])

Nosopsyllus fasciatus As summarized by Jordan [1 1] *N fasciatus*, which is of European origin, has almost become cosmopolitan but does not occur in all countries and, outside its normal range, is usually found in ports and adjacent districts According to this author it is rare in India where several similar, but distinct species of *Nosopsyllus* have been found *N fasciatus* is also rather rare in China where it is replaced by indigenous species, particularly *M anisus* As has been noted earlier, *M anisus* is also present in other countries of temperate, eastern Asia

Leptopsylla segnis *L segnis* (often called *L musculi*) is like *N fasciatus*, a palaeo arctic flea which has become almost cosmopolitan

With rare exceptions *L segnis*, when present on mice in a given locality, also infests the commensal rats sometimes to a considerable degree For instance surveys made of the fleas found on commensal rodents in *Brazil* and Malta respectively gave the following results

			Percentage incidence of			
Locality	*rats*	*mice*	*X cheopis*	*X brasiliensis*	*N fasciatus*	*L segnis*
São Paulo Brazil, 1934 [4]	90 38	9 61	21 48	38 60	rare	38 30
Malta 1936 [27]	94 10	5 80	37 50	—	13 75	48 75

Still more remarkable is the fact that Suarez [243] discovered during a flea survey at Riobamba, Ecuador that *L segnis* was the only flea species found on the commensal rats

According to Mohr,[18] *L segnis* was common on the commensal rats in the USA, but rare on *M musculus*

Other species found on commensal rodents Apart from the wild rodent fleas which are described later (see page 337), the following flea species found on commensal rodents in the various plague areas deserve attention because they have been proved or have been suspected to be vectors of *P pestis*

1 *Stivalius* species infesting rats and other rodents have been found capable of transmitting plague in Java and south India The species involved

in Java formerly called *Pygiopsylla ahalae* has since been identified as *Stivalius cognatus*[140] Several *Stivalius* subspecies seem to be concerned in south India

2 *Synosternus pallidus* a flea occurring from Senegambia and Morocco to India and infesting a variety of hosts including rodents hedgehogs and carnivora (Jordan[131]) was found to be frequent in French West Africa mainly on the floors of the houses but also to some extent on the commensal rodents It is known to bite man[1 136 141]

3 *Synopsyllus fonquerniei* a species occurring on the high plateau of Madagascar Originally identified on a small lemur and formerly apparently rare this flea has become since 1931 increasingly frequent on rats as well as on hedgehogs and insectivores (*tanrecs*)[73 8 141 17 19] According to Girard[8] it is unknown whether it attacks man

4 *Paractenopsyllus kerguisteli* a flea closely allied to *X segnis* found on the Madagascar plateau on commensal rodents and dogs but probably also infesting many other species of mammals living in forests[219 67]

5 *Xenopsylla vexabilis hawaiiensis* frequent on the commensal rodents in Hawaii where it probably plays a role as a plague vector in the fields[0 88]

6 *Pulex irritans* an Old World flea now of almost cosmopolitan distribution Though often called (or one should rather say misnamed) the human flea it has actually a remarkably wide host range In addition to infesting man and usually in small numbers the commensal rats it often attacks pigs and goats as well as foxes and badgers from which according to Jordan[131] cave dwelling man probably derived the parasite

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7 *Ctenocephalides species Ct felis felis* and *Ct canis* of palaeo arctic origin have now become almost cosmopolitan occurring not only on their specific hosts—the cat and dog respectively—but also on other species of mammals often in small numbers on the commensal rats and occasionally on other rodents Both species of fleas are prone to attack man and not infrequently become serious pests in houses

Ct felis orientis considered by Jordan[131] as the specific flea of the dog in the Orient was also found on jackals and on rodents in India[31] and in

Ceylon[104] in small numbers on the commensal rats In the *Cumbum* Valley of south India this flea was abundant on cattle, particularly buffalo calves [71]

Ct felis strongylus replaces *P irritans* in the Belgian Congo[41, 45, 60] and in Uganda as the flea of man According to Hopkins,[108] *it is abundant* on dogs and cats in East Africa and also on goats and sheep *It occurs* commonly on many other mammals of medium or large size and also not infrequently in small *numbers on the commensal rats*

8 *Echidnophaga gallinacea* an Old World flea, has now become widely distributed being not only common on poultry, but also infesting many other animals including commensal and wild rodents According to some *observers*, e g, Mohr[18] it is more frequent on *R norvegicus* than on *R rattus* It is commonly called the 'stick tight' flea, because the females, in particular, often attach themselves firmly to the head or neck of the hosts *E gallinacea* attacks man

Local distribution

It is of great importance *to realize that the commensal* rodent fleas present in any given locality often do not show a uniform distribution but display marked predilections *for particular locations* In *this connexion*, Toumanoff & Herivaux [9] aptly spoke of 'microsites' favourable for the fleas in question, but it must be kept in mind that the suitability of such sites is largely governed by the suitability of the microclimate prevailing in them

The differences in the local distribution of the various species of fleas infesting the commensal rodents may be conveniently discussed in relation to the occurrence of the latter, i e inside or outside buildings, and in special types of premises or quarters, underground, or at higher levels in urban or rural localities

General agreement exists that in contrast to species such as the palaearctic *N fasciatus* and also *X vexabilis hawaiiensis* and *S fonquerniei* *X cheopis* mainly infests the commensal rodents living inside buildings This holds true to a lesser extent of *X astia* and *X brasiliensis* because these species infest rodents other than the commensal rats and mice far more often than *X cheopis*, and are sometimes more prevalent on the wild rodent species than on the common rats and mice Davis[43] noted in this connexion that in South Africa, *X brasiliensis* was 'essentially a flea of certain veld and bush rodents that thrives in domestic premises as well'

As proved by observations in various regions e g, Ceylon, south India, Uganda and the southern areas of the USA as a rule the incidence of *X cheopis* is higher in the commercial, than in the residential quarters of the settlements, especially in premises where foodstuffs—particularly cereals—are handled or stored and in cotton warehouses and mills[101, 137]

As previously mentioned X *cheopis* is benefited by the comparatively dry situations it is apt to find in granaries and warehouses, but there are two additional factors of at least equal importance in bringing about a high incidence of this flea in locations where cereals are stored or handled namely that such establishments are attractive to rats and that cereal debris forms a most suitable pabulum for the *cheopis* larvae

The suitability of cotton for the shelterage of X *cheopis* is illustrated by observations in the Cumbum Valley [119] which showed that the incidence of this flea in rat nests containing cotton was higher than in ordinary nests

Compared to X *cheopis*, the indigenous X *astia* showed in general a more universal distribution in the settlements of Ceylon and south India It was sometimes restricted to the residential quarters, but was occasionally found to be predominant in places where rice was stored or handled

In places into which X *cheopis* had been recently imported its incidence was naturally restricted to the locations where cereals and cotton which form suitable vehicles for the transport of this flea had been stored Though subsequently an eccentric spread of *cheopis* was apt to take place this led to an invasion of the commercial quarters rather than of the residential precincts However as pointed out by King & Pandit [137] a reversal of this distribution could be observed in localities where the sanitary conditions were worse in the houses of the residential quarters than in the bazaar areas Sometimes X *cheopis* became prevalent in one part of a town whereas X *astia* remained the predominant species in the other precincts In the opinion of King & Pandit this pointed to a struggle for existence between the two species

E gallinacea was often found to be comparatively most abundant on rats in the residential or suburban zones or in rural areas particularly on animals caught near or in chickenyards and poultry houses *Ct canis* and *Ct felis* were most abundant in houses where dogs or cats were present

Dealing with the incidence of X *cheopis* and X *brasiliensis* in the settlements of Kenya Cormack [37] Roberts [214] [16] and Symes & Roberts [50] stressed that the former flea was found on rats living underground the latter on rats in roofs particularly thatched roofs Hopkins [109] considering these statements in the light of his observations in Uganda came to the conclusion that Roberts is correct in considering *cheopis* to be unsuited for life on roof dwelling rats but that *brasiliensis* can live either on these or on burrowing species

Studies on the incidence of fleas in rat nests carried out in the Salem District of Madras State also confirmed that X *cheopis* was more numerous in underground burrows than in nests found in ceilings or roofs [1 1 1] However the workers in the Cumbum Valley found that this flea did not show a predilection for any particular level [119] [1 0]

According to Hirst [10] X *astia* showed an affinity for rats living either in roofs or in burrows in the walls of domestic premises In the Cumbum

Valley on the contrary, this flea was found to predominate in rat nests recovered from underground holes [119, 120]

While in view of the discrepant findings of some workers, it is not possible to lay down hard and fast rules, the bulk of the available evidence shows that *X cheopis* has an affinity for rats living underground rather than for those sheltering on higher levels of the houses This also seems to hold true of *N fasciatus* [16]

Referring to the distribution of the commensal rodent fleas, observers in Kenya [37, 216, 0] unanimously stated that *X brasiliensis* was almost the only species present in rural areas whereas *X cheopis* was the predominant species in the mixed flea populations of urban localities

The preponderance of *X brasiliensis* in the rural areas appeared to be related to the affinity of this flea for rats living in thatched roofs, for Roberts [16] recorded that, in Pumwani, Kenya—an area inhabited by various tribes—where all the houses had iron roofs *X cheopis* preponderated at a ratio of 3 3 to 0 1 Cormack [3] seemed inclined to ascribe the preponderance of *X cheopis* in towns to the fact that many of the rats there lived underground and were then exclusively *cheopis* infested

According to Hopkins [109] *X brasiliensis* was, in general almost the only flea found on the hut rats in the rural plague areas of Uganda, while *X cheopis* was rare or even altogether absent from those parts The latter flea was common in some townships situated within the plague areas living in permanent buildings, especially shops, warehouses, and similar places In the northern, plague free zone of Uganda, *cheopis* was abundant on the house rats as well as on the field rats

X brasiliensis was also predominant in the rural areas of South Africa [4, 43] In the plague-enzootic areas of the Orange Free State in particular the rats on farms were mainly parasitized by this flea whereas in the towns and villages they were also infested by representatives of the common cosmopolitan species, that accompanied *R rattus* on its introduction, namely *X cheopis* *N fasciatus*, *N londinensis* and *L segnis* [43]

Less extensive evidence available from India [137, 75] also indicates that *X brasiliensis* is predominant in rural areas rather than in urban localities Sharif & Narasimham [239] stressed that this flea was found in large numbers on the commensal rats of localities situated in the hilly, wooded tracts of Bombay State In Dharwar town, the distribution of *X brasiliensis* was irregular although absent from one part of that community, it formed over 50% of the rat-flea population in another precinct In sections where both species occurred, certain houses were infested exclusively with either *X cheopis* or *X brasiliensis* the former fleas showing a predilection for comparatively dry and well ventilated premises, the latter being able to exist in cool, damp, ill ventilated quarters

In the experience of Sharif & Narasimham [231, 3] *X astia* was absent from the centre of the towns and comparatively most numerous in houses

on the outskirts which bordered on fields It was the predominant species on the rats of farm houses and also in premises infested by bandicoots

Wild Rodent Fleas

Annex I table V (see page 638) shows that in contrast to the commensal rodents which are infested by a few but, as a rule widely distributed flea species the groups of wild rodents found in the various plague areas are usually parasitized by diverse flea species which show almost invariably, a local or at most a limited distribution It is however important to note that as far as the individual wild rodent species are concerned, marked differences exist in this respect some of the animals having only one or two specific fleas while others have several In this connexion Eskey & Haas [51] recorded the interesting observation that the rodents parasitized by only one or two flea species usually showed a higher degree of infestation than those harbouring several kinds of fleas It is impossible to decide to what extent this is due to the presence of an antagonism between the various flea species — as is supposed to exist in the case of *X cheopis* and *X astia*—but it seems more likely that the difference is due to the advantages derived by the fleas from close adaptation to one host or a few closely related hosts

Eskey & Haas further noted that the degree of flea infestation depended also on the size of the rodents concerned These findings which seem to have been confirmed by the field observations of Humphreys et al [11] are in accord with those made in case of the commensal rats

As far as the incomplete information available goes it would appear that generally speaking in the case of the wild rodent fleas no sharp line of demarcation can be drawn between host dwelling and nest dwelling species as even those fleas which are known to have a tendency to stay on their hosts also occur in considerable numbers in the burrows of the latter This situation is well illustrated by the case of the tarabagan flea *Oropsylla silantiewi* Observations made in Manchuria and Transbaikalia [91] suggested that this flea had a marked tendency to stick to the carcasses of its hosts and to the pelts removed from them Yet Golov & Ioff [84] who had opportunities for more thorough observations in south east Russia found that even though *O silantiewi* had a propensity for staying on its hosts the majority of these fleas nevertheless dwelt in the burrows

However in the case of some flea species more clear cut differences seem to exist in this respect Thus Kusenkov [141] found that in the North Caucasus area *Citellophilus tesquorum* was found principally on the susliks while *N setosa* was found mainly in the suslik burrows The seasonal incidence of these two predominant suslik fleas was also different *N setosa* was most frequent in spring diminished in numbers in summer and became frequent once more in autumn The host dwelling *C tesquorum* on the contrary became apparently more numerous pari passu with the increase

of the suslik population due to breeding showed its maximal incidence in July, and then began to decrease

As shown by the observations of Nikanoroff & Gaiski[186] and of Borzenkov et al,[27] wild rodent fleas are not merely capable of subsisting during the hibernation period of their hosts but may even remain numerous throughout the winter However as has been established by most observers, they do not survive or stay long in uninhabited burrows, even under favourable extrinsic conditions

Eskey & Haas[54] stated in this connexion that

> the assumption has been advanced at times that fleas may continue to survive in abandoned nests and burrows for many months and if plague infected such fleas are believed to be capable of transmitting the infection over long periods Field observations however did not support this view Practically no nests yielded fleas that did not show evidence of recent occupation Even nests which were excavated within a month after ground squirrels had abandoned them were devoid of fleas

The opinion that wild rodent fleas do not survive long in burrows deserted by their hosts was shared by Kolpakova & Lippert[139] and by Fedina[81] who found that, under such circumstances the fleas sooner or later emigrated

Tiflov & Potapov[256] who also noted that the suslik fleas left empty burrows, stated that *N setosa*, while capable of surviving well on the ground outside the burrows for 24 hours, rarely survived there for 48 hours or more No doubt can exist however that wild rodent fleas may be present in large numbers on the ground near the openings of inhabited burrows Eskey & Haas stated in this connexion that by simply drawing a white cloth over the ground, they were able to collect in such a location over 200 ground squirrel fleas in a few hours

It is of interest to note that owing no doubt to the abundance of free living fleas not only in the nests of the burrows, but also in their runways and round their openings wild rodents which were released after they had been captured, marked, and de fleaed were found to be heavily re infested when caught again[106 173 231] Holdenried and his co workers were thus able to take 1 835 fleas in 37 collections from a single squirrel

Probably wild rodent fleas, although capable of staying away from their hosts in or near the burrows, are not well adapted to a free existence in more remote locations This is suggested by the observation of Vincke & Devignat[65] that, in contrast to the rat *Xenopsylla* and *Ctenocephalides* two wild rodent species—namely, *Dinopsyllus* and *Chiastopsylla*—were incapable of subsisting in debris on the surface of the ground

Interchange of Fleas between Commensal and Wild Rodents

Observations in some plague areas have shown that in locations where commensal and wild rodents could meet, an interchange of their fleas was

apt to take place As pointed out with much reason by Hopkins[109] the relative frequency of such a flea exchange indicates the degree to which contact between the two types of rodents takes place

In the western areas of the USA the occurrence of ground squirrel fleas on rats was recorded by some early observers, e g Doane[47] Fox[66] and McCoy & Mitzmain[167] Identical findings were made in the course of a recent extensive survey by Prince [09]

However Eskey & Haas[54] who examined a considerable number of ground squirrels and rats obtained from locations where these rodents intermingled found an interchange of their fleas exceedingly rare In their experience many more fleas normal to native rats and mice than fleas of ground squirrels were removed from domestic rats

Findings made during a survey undertaken on a limited scale in a rural area of California by Meyer & Holdenried[179] did not confirm the rarity of ground squirrel fleas on commensal rats because about a quarter of the species collected from these animals consisted of such fleas mainly *D montanus* On the contrary 53 ground squirrels taken from dumps and ranches heavily infested with rats yielded no rat fleas Meyer & Holdenried assumed therefore that the transfer of rat fleas to squirrels is less frequent than the reverse

As established in the course of a recent study by Holdenried[10] in the southern and southwestern areas of the USA *Polygenis gwyni* an efficient plague vector regularly infested Norway rats as well as the cotton rat *Sigmodon hispidus*

Less detailed evidence indicates that an interchange of wild rodent and commensal rat fleas also occurred in other plague areas

Thus to judge from a record by Hecht[91] *Rhopalopsyllus* subspecies were predominant on the field inhabiting rats as well as on the wild rodents examined during a survey in Aragua State Venezuela Jordan[13] examined collections of fleas from Peru and noted the occasional presence of X *cheopis* and *L segnis* respectively on some wild rodent species Ramos Diaz[211] made a similar observation of X *cheopis* on guinea pigs in the Ecuadorian Sierra

As previously noted X *brasiliensis* was found on both wild and com mensal rodents in South Africa and Uganda In Uganda[109] and in the Belgian Congo[44] this flea as well as X *cheopis* was found on the semi domestic *Arvicanthis abyssinicus* According to Heisch[9] X *cheopis* and *D lypusus* were the predominant wild rodent fleas in the Rongai area of Kenya where X *brasiliensis* appeared to be rare

X *astia* was sometimes found to be abundant on semi domestic rodents in India X *cheopis* and X *brasiliensis* were far more rarely recovered from such animals or their nests [0 71 1 3 4 31]

It will be gathered therefore that an interchange of fleas between commensal and wild rodents is by no means uncommon Fortunately

as has been discussed in chapter 6, instances where such fleas served as vectors of *P pestis* were far less frequent

Role of the Rat Flea in Plague

It is often overlooked that the credit for the discovery of the role played by the rat fleas in the transmission of plague belongs to Ogata[190] and to Simond,[33] who was obviously unaware of Ogata's publication when starting his own investigations in 1897

Working in Formosa Ogata reached on epidemiological grounds the assumption that plague was an insect borne infection and suspected the rat fleas in particular, stating that 'one should pay attention to insects like fleas for as the rat becomes cold after death they leave their host and may transmit the plague virus direct to man

Obtaining positive results in mice which had been inoculated with a suspension of triturated fleas collected from plague rats he was able to prove that these insects had actually ingested *P pestis*

As dramatically described in a paper published by Simond in 1936[31] his attention was attracted in 1897 to a possible role played by the fleas in the transmission of plague through observations on the presence of plague pustules (phlyctènes precoces) in about 5% of the patients These pustules often found on the legs of the sufferers and invariably on sound unexcoriated parts of the skin seemed to represent the portal of entry of a flea borne infection the more so as their appearance sometimes suggested that they were due to flea bites

On the other hand, Simond established that under natural conditions, infection by feeding could not play an important role in the spread of plague from rat to rat because, as shown by laboratory tests enormous numbers of *P pestis* had to be ingested to produce an infection per os and moreover the pathological findings in animals infected by this route were altogether different from those found in naturally infected or cutaneously inoculated rats

He obtained direct proof of the role of fleas in the transmission of plague by

(1) demonstrating the presence of *P pestis* in smears prepared from fleas which had been collected from plague rats and the absence of these bacilli in specimens from healthy rats,

(2) obtaining positive results with the technique used by Ogata

(3) successfully infecting healthy rats which had been confined in a wire cage and suspended above a plague infected rat in a glass jar, and failing to get positive results when conducting such experiments with rats which had been freed from ectoparasites

Finding that fleas which had ingested plague bacilli could continue to harbour them for prolonged periods Simond correctly assumed that these insects were apt to play a role in the perpetuation as well as in the transmission of the infection In fact the only one of his postulations which was not confirmed by further research was that the conveyance of plague by the fleas was invariably due to an entry of their infected faeces into the bite wounds

Though the findings of Ogata and Simond found the early support of some investigators such as Tidswell [253] and Gauthier & Raybaud,[68 69] they were in general, ignored or even ridiculed Lowe [1 3] who in 1942 published an enthusiastic appreciation of Simond s work, noted in this connexion that an editorial published in the *Indian Medical Gazette* in 1902 had spoken of the worthlessness of Simond s findings, the validity of which had been pretty completely demolished by the work of some other observers

However further evidence of the importance of fleas in the trans mission of plague was furnished by other workers e g Verjbitzki [61] and Liston [149] and from 1905 onwards a systematic investigation of this problem formed one of the principal objects and at the same time one of the monumental achievements of the Plague Research Commission As aptly stated by Wu [91]

" these classical researches still constitute the foundation of our knowledge of the transmission of plague by the rat flea Later workers have added considerably to our conception of the mechanism of infection the respective roles of *X cheopis* and *X astia* the problem of preservers and of the infective and infected flea the significance of the zoological and climatic factors and so on but their observations epoch making and conclusive though these are serve to round out the picture rather than to alter the perspective in any important particular

At the 1909 Bombay Medical Congress Lamb read a paper on The etiology and epidemiology of plague [143] which summarized a more elaborate report on the early work of the Plague Research Commission published by him in 1908 [14] and quoted the following evidence in support of the role of the rat fleas in the spread of plague

1 Attempts to convey plague from rat to rat by direct contact aerial transmission or soil infection or by means of contaminated food had shown that these modes of infection either played no role at all or did not play a role under natural conditions

2 An enormous number of successful transmission experiments had been carried out with rat fleas all other means of infection having been rigorously excluded

3 Experiments carried out mainly with guinea pigs but also with rats and monkeys in specially constructed warehouses had proved that

(a) close and continuous contact with plague infected animals (including contact with faeces and urine of the infected animals and

the eating of food contaminated with such faeces and urine) did not give rise to epizootics among the healthy animals as long as fleas were excluded,

(*b*) when fleas were present, epizootics were apt to start which varied in severity and rate of progress according to the season of the year and the number of fleas present

(*c*) the seasons in which such experimental epizootics were readily produced and spread rapidly coincided with the seasons during which plague was epidemic,

(*d*) epizootics could occur without direct contact between the originally infected and the healthy animals, the infection being effective in proportion to the access the fleas had to the healthy animals

4 The site of the primary buboes in animals found plague affected in nature and those infected in the laboratory by means of fleas was identical

5 Work carried out in houses where plague had occurred showed that

(*a*) guinea pigs allowed to run about in such houses contracted the infection in 21% of the houses, regardless of whether or not these had been disinfected with a strong solution of acid perchloride of mercury [143]

(*b*) animals which had been placed in cages to which fleas had access in plague infected houses often contracted the infection, while those exposed in flea proof cages remained healthy,

(*c*) more than 30% of the fleas trapped in plague infected houses contained abundant living and virulent plague bacilli in their gastro intestinal tracts,

(*d*) fleas derived from the carcasses of plague rats found in the houses or recovered from test animals kept in them were capable of infecting healthy animals in the laboratory

(*e*) houses which were definitely proved to be plague infected contained, on an average nearly three times as many rat fleas as houses which were only presumably plague infected and twelve times as many as houses which were free from suspicion

6 Observations on the pathogenesis of human plague and on the time relationship between epizootics and epidemics were consistent with the assumption that the rat fleas, which were found to bite man readily when hungry were responsible not only for the transmission of plague from rat to rat but also for the conveyance of the infection from the rats to man

These results are all the more striking when it is considered that the members of the Commission though believing that they worked with

X. *cheopis* only, probably also experimented to some extent with X. *astia* —a then unidentified less efficient plague vector.

Though both Simond[33] and the Plague Research Commission[702] had obtained some evidence that natural plague occurred in wild as well as in commensal rodents, the findings in the wild rodents were not sufficiently impressive to lead to investigations on the role played by their fleas. However, this gap was soon filled by McCoy[16, 166] who showed that plague could be transmitted from ground squirrels (*Citellus beecheyi*) to guinea pigs as well as between ground squirrels through one of their fleas, now called *Diamanus montanus*.

Infectibility of fleas with plague

The only way in which fleas can take up plague bacilli is by obtaining a blood meal from an animal or from a patient suffering from plague septicaemia. Fleas which ingest *P. pestis* in this manner do not contract infection in the true meaning of this term but merely become capable of harbouring this organism in their gastro-intestinal tracts. Nevertheless it is customary to consider such pestiferous fleas as being "infected" with plague. As discussed later (see page 350) a great multiplication of the ingested plague bacilli may take place in a flea's stomach and/or proventriculus and may result in a mechanical obstruction or "blockage" which sooner or later may prove fatal to the insect.

Observations by numerous workers have shown that in order to infect fleas with plague under laboratory conditions they must be exposed on experimental animals in which bacteraemia has reached a high degree. This necessity is well illustrated by the following data supplied by Eskey & Haas:[54]

Degree of septicaemia in the host (guinea pigs)	Number of fleas fed	Percentage of fleas plague-infected
10 or more organisms per field in smear	439	32
2-10 organisms per field in smear	457	29
1 or less organisms per field in smear	546	25
Smears negative, culture strongly positive	469	17
Smears negative, culture slowly positive	344	10

It will be noted that, even under optimal conditions, only about one third of the fleas used in these experiments became infected. It was found, however, that marked differences existed in this respect between the different flea species. In a series of fleas previously examined by Eskey[5] it had been established that the infection in X. *cheopis* was 66.0% as compared with about 25% in the case of *N. fasciatus*, *D. montanus* and *Hoplopsyllus anomalus* and 10% in the case of *L. segnis*.

In another paper published at the same time Eskey[51] maintained with much reason that the different infection rates observed in the various flea species might depend on differences in feeding habits, those fleas which

fed at long intervals being less likely to obtain a meal during the time their hosts had numerous plague bacilli in their blood Possibly, therefore the high incidence of infection in *X cheopis* was due to its voracious feeding habits

Findings similar to those of Eskey were recorded by Eskey & Haas [54] who, feeding 2 031 fleas of different species 3,521 times on plague infected guinea pigs, produced infection in 894 fleas They noted that practically all the 28 rat and wild rodent flea species tested could be infected to a greater or lesser extent regardless of differences in sex or size Rabbit fleas (*Hoplopsyllus glacialis affinis*) and cat fleas (*Ct felis felis*) were found much less liable to contract infection than the rodent fleas

It is difficult to decide to what extent fleas may become plague infected under natural conditions As summarized by Petrie [198] the Plague Research Commission established in this connexion that the average stomach content of a flea was about 0 5 mm^3 and that, therefore a flea imbibing rat blood containing 10,000 or more plague bacilli per ml would take at least 5 bacilli into its stomach It was estimated that 66% of the plague rats in Bombay had more than 10 000 bacilli per ml of their blood either before or immediately after their death

The Commission also established that approximately 50% of fleas collected from the carcasses of these rats harboured *P pestis*

Eskey & Haas [54] considered it as entirely problematical whether fleas were more readily plague infected under natural than under experimental conditions Since, in the laboratory the fleas were fed to capacity, their chances of ingesting *P pestis* seemed as great as when they would have fed on their infected natural hosts It seemed permissible therefore, to assume that

> the average plague infection of fleas in nature rarely exceeds one third of those feeding on their plague infected normal hosts as this was the proportion of flea infection from exposure to guinea pigs having over ten organisms per field in smears of their blood indicating about as great a bacteremia as would likely occur in any animal

Disappearance of the bacilli

As summarized by Petrie [198] the Plague Research Commission [203] found that some of the fleas which had ingested *P pestis* were able to rid themselves of the bacilli and that the clearing mechanism was probably a phagocytic one Experiments showed that a rise of temperature of about 8°C (14°F) between the limits 24°C and 32°C (75°F and 90°F) doubled the rate at which the bacilli disappeared from the gastro intestinal tracts of the fleas This seemed explained through the observation of Ledingham [145] that for a rise of 10°C (18°F) the rate of phagocytosis increased about 2 2 times

That fleas were able to get free from plague bacilli was also observed by other workers—more recently by Douglas & Wheeler [48] Eskey & Haas [54] Burroughs [5] and Holdenried [10]

Experimenting with *D. montanus* and *X. cheopis* Douglas & Wheeler noted that 62% of the *D. montanus* were free from plague bacilli 24 hours after one infective meal, while only 2% of *X. cheopis* became free after an interval of more than 48 hours.

Eskey & Haas[51] tabulated their observations as follows:

Disappearance of plague infection from fleas after existence of infection had been demonstrated by positive inoculation of faeces

Flea species	*Number originally infected*	*Percentage that became free*
Xenopsylla cheopis	140	4.0
Thrassis pandorae	58	7.0
Nosopsyllus fasciatus	51	8.0
Anomiopsyllus nudatus	9	11.0
Opisocrostis labis	178	12.0
Orchopeas sexdentatus	81	13.0
Thrassis acamantis	8	13.0
Malaraeus telchinum	74	16.0
Megarthroglossus divisus	6	17.0
Thrassis petiolatus	6	17.0
Thrassis arizonensis	58	19.0

Burroughs,[5] who carried out transmission studies with nine flea species, established that the percentages of fleas which had become free from plague bacilli at the time of their death were as follows:

Species	*Percentage*
Xenopsylla cheopis	28.0
Orchopeas sexdentatus	30.0
Opisodasys nesiotus	46.0
Nosopsyllus fasciatus	58.0
Megabothris abantis	60.0
Pulex irritans	69.0
Diamanus montanus	74.3
Malaraeus telchinum	83.0
Oropsylla idahoensis	93.0

Burroughs noted that the *X. cheopis* which were found free from plague at death had survived much longer than those in which *P. pestis* had become established.

Holdenried[10] worked with *D. montanus* and *X. cheopis* which, after an infective meal, were kept in jars at a temperature of 68°-73°F (20°-23°C) and 60%-90% relative humidity and were given opportunities to feed on normal mice. In the *D. montanus* group 25% were found to be free from *P. pestis* as early as three days after an infective meal; the last flea of this species which retained viable plague bacilli died on the 35th day. In the *cheopis* group the presence of infected fleas was demonstrated up to 52 days and only 5 out of a total of 76 fleas became free from the bacilli.

Examining fleas which had been killed at intervals of 22-31 days after infection in the course of transmission experiments, Holdenried found that

80% *Polygenis gwyni* and 68% *X cheopis* contained plague bacilli, as compared with 13% *D montanus*

Holdenried came to the conclusion that the disappearance of *P pestis* from infected fleas probably did not depend on phagocytosis which ought to have exerted an identical action in the case of all flea species He also disbelieved that the mechanical flushing out of the alimentary tract with blood ingested from normal rodents after an infective meal offered an explanation, as had been adduced He stressed in this connexion that *P gwyni* was observed to defaecate profusely after the first few feedings, yet, as noted previously 80% of these fleas continued to harbour plague bacilli until their death In his opinion therefore the mechanism by which fleas became plague free was still unknown

As shown by these observations, no doubt can exist that, at least under experimental conditions fleas which have ingested plague bacilli can become free from them It is of importance to note in this connexion that the percentage of such a clearance was markedly lower in the case of highly efficient vectors, such as *X cheopis* and *P gwyni* than in the case of less efficient vectors

Virulence of the bacilli harboured by fleas

It was suggested by some workers e g, Bacot & Martin,[8] Otten,[193 194] Meyer[1 5] Blanc[16] and Macchiavello,[156 16] that plague bacilli harboured by fleas may undergo a loss in virulence As assumed by Otten, such weakly virulent bacilli instead of producing infection, might be immunizing agents

It would be unwise to disregard the possibility that plague bacilli might become less virulent in the fleas—the more so because bacteriophage, the presence of which in *X cheopis* has been demonstrated by Girard[73] might exert an influence in this respect However it must be emphasized that in the experience of most observers prolonged survival in the fleas did not lead to an abatement of the virulence of *P pestis* As has been shown by some workers particularly Blanc,[16] and Blanc & Baltazard,[0] the virulence of these organisms is apt to remain unimpaired for prolonged periods even in dried up carcasses of plague fleas

Mechanism of Plague Transmission by Fleas

As previously noted Simond[33] when investigating the role of fleas in plague, was led to believe that the entry of infected flea faeces into bite wounds, rather than the flea bites themselves was responsible for the transmission of the infection The Plague Research Commission[0] also suspected a role of the flea faeces and suggested that a transmission of plague by fleas might be effected as well by

(*a*) the rodents eating infected fleas ,

(*b*) the proboscis of the fleas mechanically conveying the bacilli from the infected to the healthy animal ,

(*c*) a regurgitation of the stomach contents through the oesophagus and the pharynx the bacilli being then injected with the saliva or on the pricker or being rubbed into the wounds made by the pricker

These as well as some other modes of plague transmission contemplated by later investigators may be described as follows

Faeces

The Plague Research Commission paid serious attention to the possibility that the faeces of infected fleas might play a role in the transmission of plague because it had been proved by animal experiments that these dejecta contained virulent plague bacilli and also in the case of some species the fleas were apt to defaecate while feeding

As has been pointed out by later observers infected flea faeces may prove dangerous not only at the time when the fleas feed but also long afterwards because virulent *P pestis* have been found able to survive for prolonged periods even in faeces which have become quite dry [16 20 51 54 84] Likewise it was claimed that the plague bacilli voided in the flea faeces might gain entry not only through the bite wounds but also through abrasions which were pre existent or were produced when the flea bitten animals scratched themselves

However other workers maintained that for various reasons the flea faeces did not play an important role in the transmission of plague Thus Swellengrebel [16] noted that in his experience *X cheopis Pygiopsylla ahalae* (*Stivalius cognatus*) and *N fasciatus* did not defaecate while feeding The dejecta of *X cheopis* and *S cognatus* were so sticky that they adhered to the fur of the rodents instead of soiling their skins Swellengrebel was able to obtain positive results invariably when infecting guinea pigs through flea bites under precautions which excluded contamination of the animal s skins through the faeces of the fleas He felt convinced therefore that the infection was introduced by the proboscides of the fleas and not by their faeces

Swellengrebel s experiments were successfully repeated by a number of subsequent observers The viscous consistency of the flea faeces was noted also by Burroughs [5] who pointed out moreover that the posture of the fleas during the act of biting led to deposition of the faecal droplets on the hair rather than on the skin of the animals

Several workers adduced proof that the excretion of *P pestis* by infected fleas was apt to be of irregular instead of continuous occurrence Noteworthy recent observations made in this respect may be summarized as follows

Eskey stated that plague bacilli were irregularly voided in the case of some flea species Only one third of the stool specimens obtained from

N fasciatus proved positive, while those from *D montanus* were invariably positive This also held true of *X cheopis*, but no prolonged observations of individual fleas were possible in the case of this species

Eskey & Haas [54] noted that only 55% of the faecal specimens obtained from *N fasciatus* and *Thrassis francisi* gave positive results, while those from other plague infected fleas yielded 80%–90% of such results

Douglas & Wheeler [18] found that excretion of *P pestis* in the faeces of *D montanus* and *X cheopis* was quite irregular, of the daily samples obtained from these fleas 56% and 25% respectively contained viable plague bacilli The number of organisms voided in the faeces during 24 hours varied from less than 10 to a maximum of 400, with a daily average of 200

In a later publication Wheeler & Douglas [7] added that, in the case of *Hoplopsyllus anomalus* the percentage of plague positive stool samples was 63 0 as compared with 36 0 (about one third) in the case of *Ct felis felis*

According to Tiflov [34] only 50 3% of faeces samples collected from *Nosopsyllus consimilis* during periods varying from 12 to 108 days proved positive even though all the fleas were found infected upon dissection Tiflov quoted earlier observations by Golov & Ioff which gave similar, though less conspicuous results e g 33 4% negative findings in the case of faecal specimens from *Citellophilus tesquorum* In his opinion the absence of plague bacilli from the faeces by *no means* indicated their absence from the gastro intestinal tracts of the fleas in question

Burroughs [?] recorded that dead fleas were frequently proved to be infected upon their inoculation in mice, even though organisms could not be cultured from the faeces during life'

Still more important than these observations is a series of experiments recorded by Eskey & Haas [54] More than 30 attempts to infect guinea pigs by the cutaneous route with either freshly deposited or dry, faeces of fleas known to be plague infected did not lead to the infection of even a single animal

In 10 experiments the feces of 10 different infected fleas were removed from the test tubes by means of a platinum loop and a few drops of physiological salt solution and inoculated into parallel lots of guinea pigs one lot by cutaneous scarification and the other by subcutaneous injection All the animals inoculated subcutaneously were infected while all those inoculated by the cutaneous route remained entirely free from infection "

The conclusion reached by the two workers was that ' from these consistently unsuccessful attempts it may be doubted that the disease is ever contracted naturally through the agency of faecal deposits coming into contact with wounds or scratches

Infection per os

It has been maintained by some observers that plague infection may result not only from the chewing and ingestion of infected fleas (as originally assumed by the Plague Research Commission) but also by the ingestion

of their faeces or by an entry of the causative organisms contained in either fleas or faeces through the mucosa of the mouth or fauces

The natural occurrence of infections due to the swallowing of plague fleas or their faeces is altogether improbable As stated earlier, a gastro intestinal type of infection is practically absent in naturally infected rats even though they might not only swallow infected fleas or their faeces but also devour the carcasses of their infected mates

The third of the above mentioned possibilities deserves some attention on account of observations made of a rare tonsillar type of plague the appearance of which was ascribed to the habit of the people concerned of killing the vermin infesting them by biting However it is unlikely that rodents contract natural plague by contamination of the mucosa of their mouths or fauces through plague fleas or their dejecta True the preponderance of cervical buboes in plague rats might seem suggestive, at first glance of a frequent entry of the infection through the mucosa of the mouth or fauces However the total absence of any marked local reaction in these organs the occurrence of primary buboes not only on the neck but also in other parts of the body and the morbid appearances of plague rats in general are strongly suggestive of an entry of the infection through the skin and not through the mucosae Even Blanc & Baltazard [0] who succeeded in infecting one white mouse through oral installation of a suspension prepared from infected *P irritans* faeces concluded that a contamination of the mouth mucosa with the aid of such material though possible was neither easy to accomplish nor frequent

Mechanical transmission

The Plague Research Commission [0] noted that in transmission experiments carried out with numerous fleas which had fed first on plague infected rodents and then on healthy animals the largest number of infections occurred when using fleas which had been infected within 36 hours before being transferred to normal hosts These observations seemed to speak in favour of the assumption that a mechanical transmission of the infection was effected through the proboscides of the fleas which had been recently contaminated with *P pestis* However the fact that the fleas used in such mass experiments sometimes continued to be infective while feeding on healthy rats seemed to speak against an important role of a mechanical conveyance of the infection Not much attention was paid therefore to this mode of plague transmission the less so because as aptly pointed out by Burroughs [25] there soon developed a tendency to concentrate study on the individual flea rather than to work with fleas *en masse* as was previously done

The chance that manifest plague could be produced mechanically by the bite of one flea with contaminated mouthparts is altogether remote because the amount of materia morbi transmissible by the single puncture

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Eskey & Haas [54] noted that only 55% of the faecal specimens obtained from *N fasciatus* and *Thrassis francisi* gave positive results, while those from other plague infected fleas yielded 80%–90% of such results

Douglas & Wheeler [48] found that excretion of *P pestis* in the faeces of *D montanus* and *X cheopis* was quite irregular, of the daily samples obtained from these fleas 56% and 25% respectively, contained viable plague bacilli The number of organisms voided in the faeces during 24 hours varied from less than 10 to a maximum of 400, with a daily average of 200

In a later publication, Wheeler & Douglas [7] added that in the case of *Hoplopsyllus anomalus* the percentage of plague positive stool samples was 63 0 as compared with 36 0 (about one third) in the case of *Ct felis felis*

According to Tiflov [91] only 50 3% of faeces samples collected from *Nosopsyllus consimilis* during periods varying from 12 to 108 days proved positive even though all the fleas were found infected upon dissection Tiflov quoted earlier observations by Golov & Ioff which gave similar, though less conspicuous results, e g, 33 4% negative findings in the case of faecal specimens from *Citellophilus tesquorum* In his opinion the absence of plague bacilli from the faeces by no means indicated their absence from the gastro intestinal tracts of the fleas in question

Burroughs [25] recorded that dead fleas were frequently proved to be infected upon their inoculation in mice, even though organisms could not be cultured from the faeces during life

Still more important than these observations is a series of experiments recorded by Eskey & Haas [54] More than 30 attempts to infect guinea pigs by the cutaneous route with either freshly deposited, or dry, faeces of fleas known to be plague infected did not lead to the infection of even a single animal

In 10 experiments the feces of 10 different infected fleas were removed from the test tubes by means of a platinum loop and a few drops of physiological salt solution and inoculated into parallel lots of guinea pigs one lot by cutaneous scarification and the other by subcutaneous injection All the animals inoculated subcutaneously were infected while all those inoculated by the cutaneous route remained entirely free from infection

The conclusion reached by the two workers was that from these consistently unsuccessful attempts it may be doubted that the disease is ever contracted naturally through the agency of faecal deposits coming into contact with wounds or scratches

Infection per os

It has been maintained by some observers that plague infection may result not only from the chewing and ingestion of infected fleas (as originally assumed by the Plague Research Commission) but also by the ingestion

of their faeces, or by an entry of the causative organisms contained in either fleas or faeces through the mucosa of the mouth or fauces

The natural occurrence of infections due to the swallowing of plague fleas or their faeces is altogether improbable As stated earlier a gastro intestinal type of infection is practically absent in naturally infected rats even though they might not only swallow infected fleas or their faeces, but also devour the carcasses of their infected mates

The third of the above mentioned possibilities deserves some attention on account of observations made of a rare tonsillar type of plague the appearance of which was ascribed to the habit of the people concerned of killing the vermin infesting them by biting However it is unlikely that rodents contract natural plague by contamination of the mucosa of their mouths or fauces through plague fleas or their dejecta True the preponderance of cervical buboes in plague rats might seem suggestive at first glance of a frequent entry of the infection through the mucosa of the mouth or fauces However the total absence of any marked local reaction in these organs the occurrence of primary buboes not only on the neck but also in other parts of the body and the morbid appearances of plague rats in general are strongly suggestive of an entry of the infection through the skin and not through the mucosae Even Blanc & Baltazard [70] who succeeded in infecting one white mouse through oral installation of a suspension prepared from infected *P irritans* faeces concluded that a contamination of the mouth mucosa with the aid of such material though possible was neither easy to accomplish nor frequent

Mechanical transmission

The Plague Research Commission [0] noted that in transmission experiments carried out with numerous fleas which had fed first on plague infected rodents and then on healthy animals the largest number of infections occurred when using fleas which had been infected within 36 hours before being transferred to normal hosts These observations seemed to speak in favour of the assumption that a mechanical transmission of the infection was effected through the proboscides of the fleas which had been recently contaminated with *P pestis* However the fact that the fleas used in such mass experiments sometimes continued to be infective while feeding on healthy rats seemed to speak against an important role of a mechanical conveyance of the infection Not much attention was paid therefore to this mode of plague transmission the less so because as aptly pointed out by Burroughs there soon developed "a tendency to concentrate study on the individual flea rather than to work with fleas *en masse* as was previously done

The chance that manifest plague could be produced mechanically by the bite of one flea with contaminated mouthparts is altogether remote because the amount of materia morbi transmissible by the single puncture

of a flea's proboscis is infinitesimal (Hirst[9] after Walker[69]) On the contrary since it has been shown by Bychkov[30] that, under experimental conditions at least guinea pigs can become immune through the bites of plague infected fleas it is conceivable that the introduction of sub infective doses of *P pestis* on the proboscis of a flea which had recently fed on a plague affected rodent might induce a state of immunity in a healthy animal later bitten by it

However as confirmed by the recent work of Blanc & Baltazard[0] and Burroughs[5] no doubt can exist that a transmission of plague may be effected through mass attacks of fleas with contaminated proboscides

Blanc & Baltazard concluded from experiments carried out with numerous fleas that in the case of *P irritans* as well as in that of *X cheopis* one has the right to affirm that less than 24 hours after having left its dead host that is practically at its first bite of a new host, the flea is able to transmit plague to the latter[b]

Exposing laboratory rats to the bites of 60 100 *X cheopis* which had been plague infected 24 hours previously Burroughs[5] found that the animals died of acute plague 3 5 days after the introduction of the fleas i e after a time interval which disallows transmission by blocked insects as the causal factor in the transference of the organisms

Analogous experiments carried out with wild rodent fleas were frequently negative Burroughs stating in this connexion that perhaps one reason some fleas transmit more efficiently *en masse* than others is because they feed more frequently and so bite a new host animal while viable plague organisms remain on the mouthparts

Burroughs concluded with much reason that a mechanical transmission of plague through the recently contaminated proboscides of fleas was bound to be of considerable importance at the time of epizootics

Transmission through blocked fleas

As has been noted earlier the Plague Research Commission considered the possibility that a regurgitation of the stomach contents of plague fleas might play a role in the transmission of the infection No evidence to support this hypothesis seemed to be available even though Liston[150] had found in 1903 through histological examinations that after an infective meal fleas showed numerous plague bacilli in their proventriculi However, neither he nor anybody else thought at the time that these findings were related to a transmission of plague through the regurgitated gastrointestinal contents of infected fleas and it was not until 1914 that the existence and paramount importance of this mode of infection was proved through the epochal work of Bacot & Martin[8]

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[b] Aussi bien pour pulex que pour xenopsylla on est en droit d affirmer que moins de vingt-quatre heures après avoir quitté son hôte mort c est-à-dire pratiquement à sa première piqûre sur un nouvel hôte la puce est apte à lui transmettre la peste

Bacot & Martin worked with *X. cheopis* and *N. fasciatus* which had been fed on plague infected mice and which had been proved to harbour *P. pestis* by the examination of their faeces. Observing these fleas while they were feeding individually on normal rats Bacot & Martin noted that some of them merely filled their oesophagi despite their persistent attempts to suck up blood into their stomachs. Further observation showed that the reason why these fleas continued their efforts to feed was that they were in a starving condition, their stomachs and/or proventriculi having become blocked through a great multiplication of the plague bacilli originally ingested.

This condition was apt to prove disastrous not only for the fleas themselves but also for the hosts from whom they tried to obtain meals because, owing to the elastic recoil of the oesophageal and pharyngeal walls, the blood overfilling the oesophagus was apt to be driven back into the bite wounds, carrying along plague bacilli detached from the bacterial plug which was sometimes seen to protrude into the oesophagus. As shown later by Bacot, fleas which become partially unblocked through the formation of a cleft in the centre of the proventricular plug are particularly dangerous. In such cases, blood may penetrate from the oesophagus into the stomach proper but will run out again because the proventriculus, owing to the bacterial masses stuffing its lateral recesses, remains unable to fulfil its normal valve-like function, thereby preventing the regurgitation of the stomach contents into the oesophagus.

As has been demonstrated by Hirst [97] completely blocked fleas become particularly dangerous when they have fed on a host in which plague septicaemia is present because, when they attempt to feed again, such fleas will inject concentrated suspensions of *P. pestis* into their new hosts. It is obvious that the chances of such a transmission of the infection are bound to be best during active epizootics when the number of rodents in the ultimate stage of plague is apt to be large.

Studies on the process of blockage, particularly the valuable observations of Eskey [51, 53] have shown that the formation of obstructing masses due to the multiplication of the originally ingested plague bacilli may commence to take place either in the proventriculus or in the stomach proper. This is not the result of chance but of differences, probably of an anatomical nature [53, 54] existing between the various flea species. In this connexion Eskey [51, 53] and Eskey & Haas [54] noted that, in the case of *X. cheopis* the proventriculus was much more frequently the primary site of blockage than in the case of other flea species.

According to Eskey [3] a blockage originating in the stomach of a flea does not cause obstruction of that organ until it has invaded the tubular structure leading to the proventriculus and usually not until it has extended into the proventriculus or oesophagus. It is clear therefore that, because the proventriculus of *X. cheopis* is apt to become primarily obstructed

the process of blockage is bound to evolve more rapidly in this flea than in other flea species, the proventriculi of which are secondarily involved. In other words, the extrinsic incubation period, i.e. the length of time elapsing between the ingestion of plague bacilli and the establishment of blockage, is bound to be considerably shorter in the case of *X cheopis* than in the case of those fleas where blockage because it starts in the stomach proper, evolves more slowly. According to Eskey[53] and Eskey & Haas[51] *X cheopis* had an extrinsic incubation period lasting from 9 26 days, while *N fasciatus* and wild rodent fleas became blocked after intervals lasting from three weeks to over four months.

As established by these workers, the variations in the length of the extrinsic incubation period in any one flea species were due mainly to climatic influences. Eskey[52] recorded in this respect that *X cheopis* which had been kept at temperatures over 70°F (21°C) became blocked earlier than those kept at a mean temperature of 60°F (16°C) apparently because the increased temperature hastened the multiplication of *P pestis*.

Eskey & Haas[51] found that three lots of *X cheopis* which had been kept in the incubator at 72° 80°F (22° 27°C) were capable of transmitting plague at an average of 15 days after they had been infected this interval was six days less than the average extrinsic incubation period in fleas of the same species kept at 66°F (19°C) in an unheated room. Transmission by five *cheopis* kept at 80°F (27°C) occurred after they had been infected for an average of 11 days.

In the opinion of the two workers, these findings indicated that 'increased temperatures within the range of those of the experiments, should increase the rapidity with which fleas spread plague and that rodent epizootics should be more severe in warm climates than in cold, where *X cheopis* are prevalent'.

Until blockage has become established at the end of the extrinsic incubation period the fleas remain capable of feeding in a more or less normal manner without producing infection in their hosts[5]. However, it is important to note that, as established by Burroughs, *X cheopis* became blocked more rapidly and in greater numbers when kept starving for several days than when fed daily. As Burroughs pointed out this disproves the contention of Blanc & Baltazard[18, 0] that the infectivity of fleas depends upon the multiplication of *P pestis* in the fresh blood taken up at repeated feedings.

General agreement exists that though blocked fleas often remain permanently obstructed their gastro intestinal passages may become temporarily or even permanently free. Eskey & Haas[51] maintained in this connexion that temporarily blocked fleas never proved infective to the hosts on which they fed.

The survival period of blocked fleas varies according to the species concerned and also according to the prevailing climatic conditions. As a

Eskey & Haas [54] recorded the following interesting observations in this connexion

> The persistent efforts of blocked fleas to secure blood do not necessarily signify that such bites will result in the transmission of plague In fact during these experiments a great many fleas failed to transmit the infection although they fed from one to several times in a manner characteristic of complete obstruction to the stomach and only a portion of the bites of fleas that transmitted plague to one or more guinea pigs were infectious after it was apparent that blockage had developed In some instances bites of less than 1 minute s duration were infectious to guinea pigs while others lasting as long as 30 minutes failed to infect the hosts However plague transmission is more likely to follow prolonged efforts of fleas to feed than bites of short duration At least this applied to the bites made by fleas after they had infected one guinea pig for 64 percent of their subsequent blocked feeding were infectious when the parasites made multiple attempts to obtain blood while only 28 percent transmissions occurred when the insects ceased to feed after one bite During multiple bites the average attachment of the fleas was 13 minutes as compared to 4 minutes during single bites

Eskey & Haas were inclined to ascribe the frequent failure of the bites of blocked fleas to produce infection either to a lack of regurgitation or to the absence of *P pestis* in the regurgitated blood, but they also considered the possibility that in some of the fleas the virulence of the organisms might have become so attenuated as to render them non pathogenic

Since, as will be gathered from the above statements, blocked fleas are, generally speaking more likely to produce infection the more persistently they attempt to feed, it is obvious that voracious feeders like X *cheopis* will be particularly apt to act as plague vectors Observations made by Eskey [51] have proved this contention Blocked *X cheopis* repeatedly tried to feed on the guinea pigs on which they had been exposed and, in many instances, produced 2 5 foci of infection on the abdomens of the animals whereas blocked fleas of other species rarely produced such multiple foci Two *N fasciatus*, which had each infected a guinea pig, refused to bite the animals on which they were later exposed

Comparative importance of the various modes of transmission

Though as has been discussed, it has been claimed that a spread of plague through fleas may be effected in various ways it seems safe to state that only two of these modes of transmission are of real importance—namely, a mechanical spread through mass attacks of recently infected fleas, and the " biological transmission of the infection through individual blocked fleas

Comparing the importance of these two modes of transmission, Burroughs [5] postulated that

> though biologically the individual flea is all important in preserving plague in an enzootic state in areas where occasional sporadic cases of the disease occur in a sparse or highly resistant animal population mechanical transmission by numbers of infected fleas is undoubtedly the more important means of disseminating the causative organism in a dense susceptible population in epizootic times

As stated earlier one must agree that a mechanical transmission of plague by fleas is probable only during epizootics when owing to the presence of numerous severely affected rodents the infection quantum is great However, one cannot agree with the concept of Burroughs that blocked fleas are of lesser importance during epizootics because in contrast to ideas expressed by Eskey [53] Eskey & Haas [54] and Meyer [177] it fails to take into account the determinative influence exerted by variations in the length of the extrinsic incubation period Little doubt can exist that, under the climatic conditions prevailing during the plague seasons the extrinsic incubation period is short Consequently, numerous fleas become blocked and therefore infective within short periods quickly infect numerous rodents and thus create ample opportunities for a rapid further spread of the infection A vicious circle is thus set up to which momentum is added through the effect of mass attacks of recently contaminated fleas, and also through increased opportunities for a transmission of the infection by blocked fleas which have filled their oesophagi with the blood of rodents in the septicaemic stage of plague

Conclusions

As is clearly shown by these observations a marked discrepancy exists between the number of fleas which harbour *P pestis* and the percentage of those which are capable of transmitting the infection Considering the comparative infrequency of such actual vectors of the infection one must fully agree with the dictum of Webster & Chitre [7] that in the majority of infected fleas the plague bacillus has entered a blind alley and adopting the terminology used by George & Webster [71] one must realize that though many fleas may be *infected* only a minority become *infective*

Factors Influencing Role of Fleas in Transmission of Plague

Climatic influences

As has already been noted the Plague Research Commission established early in the course of its work, that the trend of experimental epizootics in rats and guinea pigs corresponded to that of the outbreaks of rat and human plague in Bombay City The experimental epizootics ran a rapid course with a high mortality during the plague seasons while the infection spread far more slowly during the off seasons and many of the animals at risk remained unaffected

That this different trend of the epizootics during the plague seasons and the off seasons respectively was due to an influence exerted by the prevailing climatic conditions on the vectors of the infection was shown through a series of well planned experiments the results of which have been summarized by Petrie [198]

> A much higher percentage of successful flea transmission experiments with Bombay wild rats and also with guinea pigs was obtained during the plague season than during the off season Experiments of this kind were made in July August 1906 that is during the off-season (1) at room temperature with an average of 27 to 28°C (80 to 83°F) and (2) in a specially constructed cool room kept at 21°C (70°F) Bombay rats ship-rats and guinea pigs were used and the proportion of successful transmissions at 21°C was nearly twice that at 27 5 C
>
> Similar experiments were made in January February 1907, that is at the beginning of the plague season (1) at room temperature namely, 24°C (75°F) and (2) in a specially constructed hot chamber kept at from 29 5 to 32°C (85 to 90°F) guinea pigs were used and the ratio of successes was 84/32, or nearly 3 to 1 in favour of the lower temperature "

In the opinion of the Commission, the failure of the infection to spread rapidly and widely at temperatures of about 80°F (27°C) or more, was due principally to a more rapid disappearance of *P pestis* from the stomachs of the fleas owing to an increased phagocytic activity of the white blood corpuscles of the host ingested together with the bacilli That fleas kept at higher temperatures were apt to become free from plague bacilli far more quickly than those kept at room temperature was shown by animal experiments with the faeces of infected fleas The dejecta of fleas kept at room temperature—24°C (75°F)—were found to be infective for 21 days while fleas kept at 32°C (90°F) excreted virulent plague bacilli for three days only

Evaluating these experiments Hirst [98] pointed out that they were open to criticism because they were probably conducted not only with *X cheopis* (as the Commission assumed), but also to some extent with *X astia* a less efficient vector and *X brasiliensis*

He further maintained that

> the growth and virulence of plague bacilli at various temperatures in the stomachs of fleas is probably dependent on a balance of several factors the rate of multiplication of the bacilli themselves a factor influenced by temperature the activity of the digestive ferments of the flea itself and the amounts secreted by its gastric glands autolytic digestion of the plague growth and the action of the ingested leucocytes

Believing that the digestive action of the tissues of the flea was apt to alter the resistance of the ingested bacilli to phagocytosis Hirst postulated that the growth of *P pestis* in the proventriculus was probably less affected by the opsonic factor than the corresponding growth in the stomach proper

While the Plague Research Commission [403] had come to the conclusion that a plague epidemic is checked when the mean daily temperature passes above 80°F [27°C] and especially when it reaches to 85°F or 90°F [29°C or 32°C] some later observers who studied the influence exerted by climatic conditions on flea borne plague postulated that dryness of the atmosphere was also of great importance in this respect

Bacot & Martin [8] maintained in this connexion that adverse climatic conditions particularly affected the blocked fleas which because they were unable to imbibe fresh blood, were in danger of drying up when the tem

perature was high and the degree of saturation of the atmosphere was low The two workers felt certain therefore that the lifetime of blocked fleas was bound to become short as soon as the weather became hot and dry, and were led to wonder whether this fact may not to some extent explain why in India epidemic plague is confined to cooler and moister seasons and particularly why in Northern and Central India the epidemics are abruptly terminated on the onset of hot dry weather

The important role played in the epidemiology of plague by the dryness of the atmosphere was fully confirmed by the exhaustive studies of Brooks [23] who found that

(1) Plague did not establish itself when the temperature rose above 80°F (27°C) accompanied by a saturation deficiency of over 0 30 inch (7 6 mm)

(2) Plague epidemics were rapidly brought to an end in the presence of a high saturation deficiency even when the mean temperature throughout and after termination of an epidemic had been below 80°F (27°C)

(3) Plague epidemics could commence and increase in intensity when the mean temperature was well above 80°F (27°C) provided that the saturation deficiency was below 0 30 inch (7 6 mm) [c]

In the opinion of Brooks the adverse influence of high temperature and saturation deficiency may be explained by their effect on the duration of life of the rat flea *Xenopsylla cheopis* when separated from its host

Goyle [8] studying the influence of climatic conditions on *X cheopis* and *X astia* came to the conclusion that at a temperature of 68°F (20°C) *X cheopis* was incapable of transmitting plague at a saturation deficiency of 0 6 inch (15 2 mm) while a saturation deficiency of 0 3 inch (7 6 mm) at the same temperature was sufficient to check the conveyance of the infection by *X astia*

Webster & Chitre [5] who published soon afterwards the results of large scale transmission experiments carried out with these two flea species and also with *X brasiliensis* obtained evidence to support the view that

> " the chief factor in the spread of plague which is under the influence of climatic changes in Bombay is connected with the blocking phenomenon The epidemic season seems to be controlled by the fact that within a rather limited range of temperature [68 8 76 8 F (20 5 24 9 C)] and humidity [saturation deficiencies ranging from 0 237 inch to 0 374 inch] a much larger proportion of infected fleas became capable of transmitting the infection

These observations tally with those of Eskey [5] and Eskey & Haas [51] on variations of the extrinsic incubation period at different temperatures

As is to be expected findings analogous to those discussed above were made when instead of variations of the saturation deficiency of the atmosphere those of the relative air humidity were observed Thus Campbell [3]

[c] Otten [illegible] added [illegible] that these conclusions [illegible] where an increase of plague was noted during the dry [illegible] of the year and a decrease during the wet season

noted that a plague outbreak at Mwanza, Tanganyika started, and ran its course, at a relative humidity of 80% and came to an end two weeks after the relative humidity of the air had become less Robic [317] ascribed the rapid cessation of plague outbreaks in Madagascar at about 30°C (86°F) to variations in the relative humidity which were apt to occur at that level of temperature and which impaired the resisting power of *X cheopis*

While as will be gathered from the evidence quoted, general agreement exists that climatic conditions exert a profound influence on the role of fleas in the transmission of plague, vastly different views are held by the different observers as to how this influence is exerted It is claimed by some workers that changes in temperature and humidity are of importance because such changes exert an influence on plague infected fleas, while other observers maintain that these climatic factors influence the blocked fleas in particular

It is undeniable that variations of the temperature and humidity may regulate to some extent the number of fleas which continue to harbour *P pestis* in their gastro intestinal tracts However, even under the most favourable climatic conditions, only a minority of these infected fleas become infective, and variations in their numbers, unless they become very marked, are probably not of great epidemiological importance However, it is of paramount importance that favourable temperatures, because they shorten the extrinsic incubation period lead to a rapid increase of the number of blocked fleas, and that favourable humidities are apt to increase the length of survival of these infective fleas One must therefore agree with the statement of Park [195] that 'summing up the findings on plague transmission by fleas it appears that it is not the total number of fleas or the specific flea counts that matter not even the number of infected fleas but the number of infective fleas that is of prime importance, and this depends perhaps entirely on climatic conditions

However, it is of the greatest importance to remain aware of the fact that because the microclimate in the rodent burrows is apt to remain uniform throughout the year it may continue to be favourable for a transmission of plague through the fleas even during seasons when the generally prevailing climatic conditions are unfavourable for a spread of the infection

Vector capacity

The existence of differences in vector capacity between the various flea species was established early in the work of the Plague Research Commission and this problem has engrossed the attention of numerous workers ever since The methods used for a study of this question, although considerably varying in details may be classed in two categories

(1) Mass transmission tests carried out by the exposure of groups of fleas previously fed on a plague infected animal on healthy laboratory animals the fleas usually being put on the healthy animals a few days

after the original host had died so as to exclude the possibility of a mechanical conveyance of the infection

(2) Individual feeding experiments i e those carried out with single or a few plague infected fleas

With regard to the value of these procedures it should first be stated that both methods possess a common drawback—namely, that for various reasons particularly in order to obtain comparable results when testing different flea species it is often necessary to work not with their specific hosts but with highly and uniformly susceptible laboratory animals As aptly pointed out by Burroughs [5] this drawback is of little importance in the case of some flea species e g *X cheopis* for which favourable conditions may be created in the laboratory It is however apt to vitiate results in the case of flea species which normally live in a cold climate or when dealing with strains of species such as *P irritans* which have become adapted to mammalian hosts other than rodents

A great advantage of mass transmission experiments is that they may be conducted under conditions resembling to quite some extent, those in nature In fact by adding at suitable intervals new healthy animals to those initially introduced into the experimental cages warehouses or pits veritable epizootics may be established and kept going However while valuable for epidemiological studies mass transmission experiments cannot be used to determine the vector capacity of fleas in an exact manner Nevertheless it is advantageous to use them before individual feeding tests are carried out for this purpose so as to arrive at a preliminary estimation of the vector capacity of the species to be examined

Individual feeding tests as usually performed possess the drawback of artificiality because whereas the fleas in nature have as much access to their hosts as they feel impelled to seek during these tests they are permitted to bite or to try to bite merely during limited periods and usually only once daily In order to obtain comparable results when various flea species are examined this procedure must inevitably be followed However it must be kept in mind that the method does not necessarily give a complete picture of the capability of the individual species to transmit plague under natural conditions

The following recently recommended methods to determine the vector capacity of fleas deserve mention

Wheeler & Douglas [6] first carried out tests en masse with each flea species to be examined in order to determine whether it was able to transmit *P pestis* For this purpose 100 fleas of the species in question were fed on a guinea pig or a white mouse in the ultimate stage of plague Two days after the death of the infected host the surviving fleas were placed on a healthy guinea pig or were allowed to feed on healthy mice

For individual feeding tests Wheeler & Douglas used whenever possible laboratory reared fleas which were fed on a moribund plague infected

mouse whose degree of septicaemia had been controlled Some hours after the death of the mouse, the fleas were recovered, placed in a test tube, lightly anaesthetized with ether, and examined under a dissecting microscope Fleas which had not obtained a complete blood meal were discarded The others were placed individually in 75 mm by 15 mm glass shell vials the mouths of which were closed by a square of fine mesh, silk bolting cloth held in place with a rubber band, and were kept at a temperature of 20°-23°C and an average relative humidity of 50%

By inverting the vial and placing the open end securely against the shaved abdomen of an immobilized mouse, each flea was given a daily opportunity of feeding upon a healthy mouse After feeding each flea was transferred to a fresh, sterile vial and the faeces contained in the used vial were cultivated At death, every flea was fixed and sectioned serially for histological studies

The mice on which the infected fleas had fed were placed in individual glass cages and observed for a period of 30 days Each animal dying within this period was dissected, and smears and cultures were made to ascertain the presence of *P pestis*

The information obtained by the examination of the fleas and the mice was used to establish (1) the infection potential, based upon the percentage of the fleas of a given species in which *P pestis* had become established (2) the vector potential, based upon the percentage of infected fleas capable of transmitting plague and (3) the transmission potential i e the mean number of transmissions effected by the infective fleas The product of these three factors represented the number of transmissions effected by a given number of the fleas, or the vector efficiency

Wheeler & Douglas [76] thus illustrated the manner in which these potentials were established

> Individual feeding trials have demonstrated *D montanus* to have an infection potential of 85 a vector potential of 52 and a transmission potential of 2 58 with a vector efficiency of 1 14 That is of 48 fleas used 41 or 85% became infected Of these 41 infected fleas 21 or 52% became infective and transmitted the infection to 50 mice or an average of 2 58 transmissions per infective flea Dividing the percentages by 100 the potentialities of a single flea may be obtained In other words a group of *D montanus* given an infectious blood meal will yield an average of 1 14 transmissions per flea "

The results of the tests made with *D montanus* and *X cheopis* were as follows

	D montanus	*X cheopis*
Number used	48	50
Number infected	41	49
Number infective	21	14
Number of transmissions	50	20
Infection potential	0 85	0 98
Vector potential	0 52	0 29
Transmission potential	2 58	1 44
Vector efficiency	1 14	0 39

Wheeler & Douglas noted that, according to these results *X. cheopis* had a higher infection potential than *D. montanus* but that only half as many *cheopis* became infective and transmitted only half as many times. They concluded therefore that

> apparently then a high infection potential alone is no indication of high vector efficiency; likewise the vector potential alone without a consideration of the transmission potential will not give a true picture of vector efficiency. From the above data an accurate evaluation of vector efficiency must include a knowledge of the infection potential, the vector potential and the transmission potential.

In a second paper Wheeler & Douglas [77] recorded the results of further experiments made with *D. montanus* and *X. cheopis* as well as with *N. fasciatus*, *Ct. felis felis* and *Hoplopsyllus anomalus*. All five species were found capable of transmitting *P. pestis* in mass experiments. Results of individual transmission trials in four of these species showed the following vector efficiencies:

Species	Vector efficiency
Ct. felis felis	0
H. anomalus	0
X. cheopis	0.43
D. montanus	0.84

Using techniques which were similar to, though more refined than, those of Wheeler & Douglas, Burroughs [5] compared the vector capacities of the ten flea species studied by him by ascertaining the transmission rate of each species, i.e. the quotient obtained on dividing the number of transmissions effected by the fleas in question, when given one daily opportunity to feed, by the total number of fleas. His results were as follows:

Species	Number tested	Number of transmissions	Ratio of transmissions to fleas used
X. cheopis	53	35	0.660
E. gallinacea	48	12	0.250
N. fasciatus	47	10	0.213
O. s. sexdentatus	53	9	0.170
O. nesiotus	46	3	0.065
M. abantis	75	4	0.053
M. telchinum	115	5	0.043
D. montanus	66	1	0.015
P. irritans	57	0	–
O. idahoensis	61	0	–

As stated by Burroughs,

> "... the transmission data obtained from individual flea feeding studies was analysed statistically to estimate the expected number of transmissions per flea of each species. These values are obtained as intervals which have a 90% probability of containing the true value. The true vector efficiency of *Xenopsylla cheopis* was found to be 0.660 ± 0.234 (expected transmissions per flea), that of *Nosopsyllus fasciatus* to be 0.213 ± 0.157, and that of *Orchopeas sexdentatus sexdentatus* to be 0.170 ± 0.138. *Opisocrostis nesiotus*, *Megabothris abantis*, *Malaraeus telchinum* and *Diamanus montanus* transmitted very inefficiently."

Devignat[41] made mass transmission experiments in order to determine the vector capacity of the important flea species found in the Belgian Congo He found that the constants of vector capacity thus obtained equalled either 1 or 0 However, differences in the vector capacities of the fleas found able to transmit plague could be determined by comparing the surface areas of the curves illustrating the mortality of the flea bitten mice with the surface area of a graph showing the mortality of mice infected with standard doses of *P pestis* which was equal to 7 600 mm He tabulated his results as follows

Flea species	*Surface area of graph showing mortality of flea bitten mice (mm²)*	*Ratio to surface area* of graph showing results of virulence tests*
Xenopsylla cheopis	7 300	0 96
Xenopsylla brasiliensis	5 400	0 71
Dinopsyllus lypusus	6 900	0 90

* 7 600 mm

Investigating the problems of flea borne plague, some workers were led to believe that the capabilities of male and female fleas respectively of transmitting the infection were unequal Hirst[101] recorded in this connexion that all the fleas which he had found to be capable of conveying plague were females On the contrary Goyle[87] postulated that male *X cheopis* and *X astia* carried the infection more readily than the females of these species

The results obtained in this connexion by Webster & Chitre[74 2 3] were discrepant They concluded from some of their early experiments that female *X cheopis* and *X astia* were more capable transmitters than the males Reporting on further investigations, they stated that

> the question of the sex of the flea in connection with its transmitting power is not easy to answer From the mixed flea experiments it would have seemed reasonable to conclude that of the six groups male *cheopis* and male *brasiliensis* are much the most regular transmitters With the pit fleas however the cage experiments show female *cheopis* and female *astia* to be much more effective than their respective males Male *astia* fleas have been found of low value as transmitters in all the experiments The finding of an individual blocked male *astia* capable of infecting with a single bite was particularly fortunate

Eskey[51] stated that, though he performed experiments with many males of different species, only two of the 45 fleas which he found capable of transmitting the infection were males

The results of the experiments made by Eskey & Haas[54] were similar to those of Eskey, but not as clear cut Moreover as shown by the following figures, the number of female fleas observed was markedly in excess of the number of male fleas

	Females	*Males*	*Total fleas*
Number plague infected	607	243	850
Number that were vectors	70	11	81
Percentage infected that were vectors	11	4	9

Wheeler & Douglas [7] recorded the following observations:

	X. cheopis			*D. montanus*		
	males	*females*	*total*	*males*	*females*	*total*
Number used	2	47	49	18	62	80
Number infected	2	45	47	12	55	67
Number infective	1	13	14	2	30	32
Number of transmissions	1	20	21	3	64	67
Infection potential	1.00	0.96	0.96	0.67	0.89	0.84
Vector potential	0.50	0.29	0.30	0.17	0.54	0.48
Transmission potential	1.00	1.54	1.50	1.50	2.13	2.09
Vector efficiency	0.50	0.43	0.43	0.17	1.02	0.84

It will be noted that, as far as these figures go, no marked differences in vector efficiency appeared to exist between the sexes in the case of *X. cheopis*. Male *D. montanus* were found to be less efficient vectors than the females, but only 18 males were studied as compared with 62 females.

Holdenried [10], testing 39 male and 49 female *Polygenis gwyni*, found that 11 (28.2%) males were transmitters as compared with 13 (26.5%) females.

In view of the fact that the various observers quoted above often used unequal numbers of male and female fleas respectively, and considering that even their total samples were not large, one ought to accept the statement of Webster & Chitre [74] that possibly "a larger series of experiments under any one set of conditions would abolish the apparent difference in the value of males and females as transmitters".

A question of great interest and possible importance is whether the vector capacities of different strains of the same flea species may be at variance. It is striking to note in this connexion that, as previously mentioned, *D. montanus*, while proving in the experience of Wheeler & Douglas [6, 7] an even more efficient vector than *X. cheopis*, figured last among the transmitters given in Burroughs' list [5]. Results similar to those of Burroughs were recorded by Eskey & Haas [51], who noted that out of 19 *D. montanus* tested only 3 transmitted plague, and by Holdenried [10], who found that out of 446 fleas of this species only 9 conveyed the infection.

Discussing this problem, Burroughs [5] was inclined to believe that the discrepancy between his results and those obtained by Wheeler & Douglas was due to biological differences between the strains used in the two experiments. There was no doubt that the fleas used by Wheeler & Douglas came from a locality different from that where Burroughs obtained his material.

The contention of Burroughs was supported by the observations of Holdenried [105], who found that three of his *D. montanus* strains obtained from different plague foci as well as a fourth strain from an apparently plague-free region yielded no vectors at all, whereas strains from two other supposedly plague-free localities yielded vectors at a rate of 4% and 5% respectively.

for instance particularly infests rats in grain stores and is markedly more abundant on rodents trapped inside buildings than on those caught outdoors

(8) Fleas do not uniformly infest all rodents of a locality, but show a patchy distribution so that sometimes a large part of a flea population is concentrated on a few animals

Although it is desirable to do so it is rather difficult to make exact determinations of the number of rodents in the course of routine flea surveys as recommended by Cole & Koepke [35] However advantage may be taken of a simple method recommended by Hirst [103] The number of rodents caught per 100 traps set is ascertained and this factor is multiplied by the flea index figures As stated by Hirst in this way an index representing the gross ecto parasitic flea population is obtained

In order to eliminate the errors arising out of an abnormally heavy infestation of a few rodents Rumreich & Wynn [1] recommended the adoption of a new index derived by mathematically fitting an appropriate curve to the frequency distribution of parasite counts in any host population and thus determining their normal upper limit However as pointed out by a reviewer in the *Tropical Diseases Bulletin* [60] an index computed in this way loses its great value of being able to be compared for different places

To improve the value of index determinations Cole & Koepke recommended that the indices should be computed for flea infested rodents only and that male *X cheopis* should be disregarded in such computations because as shown by Cole [34] the females of this species are less variable than the males in their association with the hosts under varying temperature conditions

In the opinion of some workers more reliable results could be obtained in flea surveys by computing instead of the indices the percentage incidence of the various species or the infestation rates i e the percentages of rodents infested with the various flea species

Hirst [10] maintained with much reason that computations of the percentage incidence of fleas could be legitimately used only when the general flea index was more or less uniform because

" suppos the average number of all spe ies of fleas per rat in one locality is two and in another twenty and suppose there are 10 per cent *cheopis* in a parallel collection from each then the *cheopis* index in the first case is 0 2 and in the second 2 0 If the mean temperature is below 80 F [27 C] the second figure is compatible with a severe epidemic whereas a serious outbreak would be unlikely in the first instance "

It follows that in order to obtain fully reliable results it is best to determine both the indices and the percentage incidences of the various flea species as has been recommended by King & Pandit [13]

Discussing observations made during a survey at San Francisco Calif Eskey [5] found that the *cheopis* index fluctuated so erratically from month

Considering the limited scope of these observations, one must agree with Holdenried that the evidence concerning the existence of racial differences in the vector capacity of fleas belonging to the same species, although suggestive, is not fully convincing

Vector incidence

Before appreciating the influence exerted on the transmission of plague by variations in the incidence of the vector fleas, it is necessary to consider the methods recommended for flea surveys

The early workers, though realizing the ominous role of the fleas which had left the carcasses of plague infected rodents paid no attention to these loose parasites when conducting routine surveys but relied for this purpose, on flea collections from rodents which had been trapped alive They used this material to determine for each rodent species, separately, (*a*) the total flea index i e, the average number of fleas of all species per rodent, and (*b*) the specific flea indices, i e the average number of fleas of each species found per rodent

Though ample use was, and is, made of these procedures, it was pointed out by some observers particularly by Hirst,[100, 101] and Cole & Koepke[3] that many variables coming into play were apt to detract from the value of flea index determinations Cole & Koepke stressed in this connexion, that

(1) Indices computed from total fleas are unreliable because they are apt to include non vectors as well as species which are unequally effective as vectors

(2) The time fleas of different species spend on their hosts is apt to vary considerably

(3) No reliable measurements of the absolute number of the fleas can be made without considering the number of rodents because a decreasing host population will concentrate the ectoparasites and raise the mean number per rat while an expanding host population will reduce the average counts

(4) The host species ought to be uniform in compared samples because, as in the case of *R norvegicus* and *R rattus*, for instance different rodent species show differences in flea infestation

(5) The age as well as the size of the individuals affects the counts young and old rodents being liable to heavy infestation The same may hold true of unhealthy animals

(6) Trapping techniques must be consistent in order to obtain comparable data

(7) Widely different indices may be obtained from rodents trapped in different sections of a town or in different locations within one particular neighbourhood or even on different levels of one building *X cheopis*

for instance particularly infests rats in grain stores and is markedly more abundant on rodents trapped inside buildings than on those caught outdoors

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to month that it was of little value for determining the extent of seasonal infestation ' He therefore recommended that reliance should be placed upon the percentages of specific flea infestation Rumreich & Wynn [1] although establishing that there was a marked parallelism between these values and the specific indices, recommended the use of both methods in order to assess the incidence of the various flea-species

Within recent years it has been urged by several workers that, in addition to surveys carried out with the aid of such methods the incidence of free living (loose) and burrow- or nest dwelling fleas ought to be assessed

Various methods have been recommended for the collection of loose fleas, including the exposure of guinea pigs on the floors of houses or of guinea pigs or de fleaed ' rats in cages, the use of flea traps, and the use of adhesive paper to which the fleas could become attached [01, *, 6]

A simple and efficient trap recommended by Lefrou & Wassilieff [148] consisted of a dish filled with oil and provided with a floating wick which was lighted at night to attract the fleas However it was found sufficient to fill the dishes with water preferably adding a little alcohol or chloroform Nowadays a DDT suspension or solution might be substituted with advantage

It was originally recommended that the trap should be used in houses with a soft floor so that the rim of the dish could be made level with the ground However, as shown by M A Farid (unpublished observations in Lebanon), numerous fleas could be caught by placing 2 5 cm high lighted traps on the ground The provision of a 7 5 cm wide sloping rim, the outer margin of which was made to rest on the ground to enable the fleas to crawl up instead of jumping hardly increased the size of the catches

A suitable apparatus for collecting fleas which harbour in dust has been described by Estrade [55] as follows

The apparatus consists of (*a*) a large truncated cone, in the centre of the inside bottom of which a plate with a pin is affixed, and which is filled with a layer of water, flytox, or alcohol before use, (*b*) a funnel, of hour glass shape, closely fitting over, (*c*) a receptacle with a 2 cm high outer wall and a hole in the centre of its bottom

The dust to be tested is rapidly filled into the upper mouth of the funnel which together with the receptacle underneath is placed into the large container in such a way that its central pin engages with the receptacle The funnel is then removed, contact thus being established between the dust and the fluid in the container As soon as this happens the fleas in the dust begin to jump and land in the fluid where they are drowned

Most workers resorted to digging operations in order to obtain fleas from the passages and nests of rodent burrows However, some investigators [5, 61, 4] caught wild rodent fleas with the aid of cotton wads inserted into the mouth of the burrows or by trapping For this purpose workers in south east Russia used a trap consisting of a metal box filled with water

and provided with a tube the open outer end of which was inserted into the opening of the burrow [6] Pasteboard tubes smeared inside with glue as used by Traut (quoted by Wu Lien-teh & Pollitzer [91]) seem to have given less satisfactory catches [d]

Conclusions of the composition of the flea populations examined with the aid of these methods may be reached by establishing the percentage incidence of the various species of loose or burrow fleas found It is important to note in this connexion that according to Stewart & Evans [1] the composition of the flea population at the openings of wild rodent burrows corresponds quite accurately though on a lesser scale to that of the flea material collected from the rodents themselves Stewart & Evans therefore considered it legitimate to make surveys by studying the fleas obtained from the openings of ten or more burrows It is not certain however whether this conclusion is generally valid

Some recent workers particularly Davis [41] and Macchiavello have recommended that the total or absolute flea index should be determined by considering the results of examinations of the burrows and nests as well as those of surveys carried out in the usual manner Macchiavello recommended the application of the formula $AFI = \frac{RF + NF}{TR}$ where AFI stands for the absolute flea index RF for the total flea population living on the rodents NF for the absolute number of fleas from flea breeding or flea harbourage and TR for the total rat population of the area concerned These data were to be obtained by cross section surveys of rodent and flea populations similar to those used in the partial census of human population and a cross section counting of nests in a representative limited area [29]

Desirable as determinations of the absolute flea indices by this or other methods are it seems difficult if not impossible to make routine use of such procedures

The results of flea surveys carried out in the usual manner are of great value since they permit an assessment of the plague situation It is generally accepted that as has been postulated first by Hirst [100] and later by Grubbs [89] even though the infection is present or is introduced there is no danger of an epidemic spread as long as the *cheopis* index remains below 1 Of course this applies only to areas where *X cheopis* is the sole important vector

To judge from less detailed information it would also appear that plague is not liable to assume epidemic proportions as long as the *cheopis* percentage is below 20%–30% [26a] or as long as the *cheopis* infestation rate is below 30% [50]

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[d] A further method used by George & Webster is described on page 381

It is however, of the greatest importance to realize that the se during which plague outbreaks occur do not necessarily coincide wit periods during which the incidence of the vector fleas is highest Kitasat stated in this connexion that in Japan the rat fleas were most preva during summer, although the plague season occurred in the cold per of the year

Similarly it was found by Eskey & Haas [54] that the percentage plague infected ground squirrels in the vicinity of San Francisco Bay wa highest during the three summer months when the flea infestation of th animals was lowest

George & Webster [71] concluded from their observations in the Cum bum Valley of south India that

> the *cheopis* index and plague incidence are not so closely associated as appears at first glance Thus in 1931 the decline of plague in February preceded the fall in the *cheopis* index while in the autumn epidemic the plague incidence increased prior to the increase in fleas In 1932 the order of events was the same but to a less marked extent In 1933 the high plague incidence in the spring was not associated with a corresponding rise in the flea index figure while the autumn rise in the *cheopis* index was not accompanied by an increased incidence of plague

Similar observations were repeatedly made by Pollitzer in central China

Robic [17] stated that in Tananarive, Madagascar there was no definite relation between the *cheopis* index and the frequency of plague while Herivaux & Toumanoff [93] found that the *cheopis* index at Saigon, Indo china, was highest when plague was absent

Webster & Chitre [275] maintained in general that ' the plague season need not correspond with the season of maximum prevalence of rat fleas although this may happen as a coincidence

These observations lend strong support to the contention that it is a high incidence of actually infective fleas and not the frequency of poten tially dangerous vector species, which is of paramount importance in the spread of flea borne plague

Role of Individual Flea Species

Rat Xenopsylla

Though the investigations of the Plague Research Commission estab shed the paramount importance of the *Xenopsylla* in the transmission rat caused plague they failed to render full justice to this subject since e members of the Commission laboured under the assumption that *cheopis* was the only representative of this genus of flea found on the mensal rats of India It is to the great merit of Hirst that he was able rove (through exhaustive studies which were commenced in 1912 and hich the first results were published in 1913 [94]) that in addition to

cheopis two other *Xenopsylla*—namely *X. astia* and *X. brasiliensis*—were present on the rats.

Hirst, finding that *X. astia* was a far less efficient plague vector than *X. cheopis*, propounded the idea that the preponderance of this flea in some parts of India offered an explanation of why these localities were free from the infection. This concept was not in accord with the views of the Plague Research Commission [91] and Liston [150] who ascribed the absence of plague from such localities—particularly from Madras City—to climatic conditions unsuitable for the spread of plague. It is not surprising, therefore, that Hirst's statements were received with a great deal of scepticism at first. Petrie [198], for instance, maintained as late as 1929 that the question of the relative importance of *X. cheopis* and *X. astia* in the spread of plague was still unsettled and that, therefore, further work on the subject was necessary before final conclusions could be drawn.

However, the findings of Hirst, published in a series of studies [9, 101], were confirmed on the whole by the laboratory investigations of Taylor & Chitre [1], Goyle [8], Webster & Chitre [4, 5] as well as by the results of extensive field surveys recorded by Cragg [39] and by King & Pandit [13]. General agreement has thus been reached that, although *X. astia* is capable of becoming blocked and of transmitting plague, it is a markedly less efficient vector than *X. cheopis* or *X. brasiliensis*. Webster & Chitre [5] stated in this respect that

> the exact numerical value of the different species cannot yet be laid down. The renewed epizootic in the *astia* pit provided some information regarding this species. With a flea index of even the epizootic could be restarted. When the epizootic had ceased the flea index was found to be 3.2. Under the most favourable conditions the necessary *astia* index may lie between these two figures. In nature a still higher index is probably required. The mixed flea experiments in the plague season, taking both sexes of each species and counting the first transmission in series only, give the relative value as transmitters of *cheopis*, *astia* and *brasiliensis* as 1 : 0.3 : 1.7 respectively.

These conclusions were endorsed by findings made in the course of field surveys. King & Pandit [13], summarizing the results of a rat flea survey in Madras Presidency (now Madras State), stated that "the occurrence and severity of plague epidemics are associated with the number of *cheopis* present and this relation is mainly direct and not just because of the common association of plague and *cheopis* with climate" and that "*X. astia* acting as vector in nature without *cheopis* in south India has produced very few and very small epidemics which did not carry over the off season".

Hirst [104], reporting on a rat flea survey of Ceylon, concluded that

(1) In Colombo nearly two thirds of the plague manifestations detected in rats during the period 1918-29 occurred in the main *cheopis* area, while the far more extended *astia* zones, which contained a few *cheopis*-infested premises, yielded only one tenth of the positive rats.

It is however, of the greatest *importance to realize* that the seasons during which plague outbreaks *occur do not* necessarily coincide with the periods during which the *incidence of the vector fleas is highest* Kitasato[138] stated in this connexion that in *Japan*, the rat fleas *were most prevalent during* summer although the plague season occurred *in* the cold period *of the year*

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"... the exact numerical value of the different species cannot yet be laid down. The renewed epizootic in the *astia* pit provided some information regarding this species. With a flea index of seven the epizootic could be restarted. When the epizootic had ceased the flea index was found to be 3.2. Under the most favourable conditions the necessary *astia* index may lie between these two figures. In nature a still higher index is probably required. The mixed flea experiments in the plague season, taking both sexes of each species and counting the first transmission in series only, give the relative value as transmitters of *cheopis*, *astia* and *brasiliensis* as 1 : 0.3 : 1.7 respectively."

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(2) Out of 11 rat epizootics detected in Ceylon outside
eight occurred in districts with a *cheopis* index of 1 0 in one distr
was present, but the index was not determined while two epizooti
in a pure *astia* area at Galle where the *astia* index was 5 67

(3) A total of 25 human cases resulted from these two
which did not spread beyond the original focus of infection
were separated from each other by a clear interval of seven years

(4) Evidence from Ceylon and Madras combined with ex
data suggested that the critical *astia* index for independer
transmission lay between 6 and 7

(5) Analysis of the Colombo data suggested that, when an
is invaded by *cheopis* plague may become endemic when the *che*
is near unity the additional *astia* apparently playing a subsi
once the epizootic has been initiated by *cheopis*

Although these findings confirm the paramount importance
efficient vector species in the transmission of plague they show
efficient vectors if present in sufficiently large numbers may pl
sidiary or even, to some extent, an independent, role

The laboratory observations made by several workers e g, \
Chitre [4 275] Symes [318] Roberts,[215] and Devignat,[44 45] have pr
X brasiliensis is an efficient plague vector, in fact as already notec
& Chitre [97a] *found the vector capacity of this flea to be superi*
of *X cheopis*

Davis [4] stated that while no exact laboratory data had been
on the vector efficiency of the fleas involved in the South Afric
outbreaks *X philoxera* (*X eridos* auctt)—the gerbil flea—and
liensis were regarded, on epidemiological grounds as equally
vectors of the infection to rodents and man *X brasiliensis* was
responsible for the majority of human infections particularly in
areas where it lived closest to man

Hopkins [109] considered *X brasiliensis* as the normal initiat
plague outbreaks in Uganda In his opinion *X cheopis* was ver
concerned in the initiation of outbreaks but helped to carry on
which had reached townships

Dealing with the situation in the Lake Albert plague focus of th
Congo Vincke & Devignat [265] stated that though both *X che*
X brasiliensis had been found infected, the percentage incidence of
was invariably high (20% 30%) whereas sometimes less than 10%
liensis were found Even in the zone where *X brasiliensis* pred
in general, *X cheopis* was found to be prevalent in some of the pla
In the opinion of Vincke & Devignat, *X cheopis* was a more efficie
as far as the transmission of plague from rodent to rodent was co
whereas *X brasiliensis* was particularly apt to convey the infection

Therefore an optimal mixture of both these flea species might lead to the appearance of human plague as well as of epizootics

According to Riel & Mol[213] X brasiliensis was the vector of plague in the Lake Edward plague focus of the Belgian Congo from which focus X cheopis was absent

As stated by Roberts[14, 216] X brasiliensis was the principal vector of *P pestis* in the rural areas of Kenya where plague was endemic in type whereas X cheopis was mainly, or even solely involved in the urban manifestations of the infection which were of an epidemic character Roberts ascribed this different role of the two flea species to differences in their habitat X cheopis mainly infested the rodents living underground in the houses and thus had far more easy access to human beings than X brasiliensis, the latter was found mainly on the rodents which sheltered in the thatched roofs of rural buildings but did not actually enter the premises

Similarly it was maintained by Sharif & Narasimham[23] that X brasiliensis because of its preference for the hilly woody tracts of the Western Ghats in Bombay State played a principal role in the plague outbreaks occurring in these localities which showed a marked tendency to spread slowly but to persist for a long time X cheopis was probably a more important vector in the low comparatively warm tablelands of Bombay State where the plague outbreaks were of an explosive nature but did not last long

Apparently *X brasiliensis* because it was most prevalent during the plague season played a more important role than *X cheopis* in the transmission of the infection in the Salem District of Madras State[1, 3]

In addition to playing a predominant role in the above mentioned areas *X brasiliensis* is held to be an important plague vector in the temperate zone of Brazil especially in São Paulo[15, 207]

Eskey[50] assumed primarily on epidemiological grounds that in addition to *X cheopis* *X vexabilis hawaiiensis* played an important role in the transmission of plague in Hawaii

Rat Ceratophyllinae

In 1907 the Plague Research Commission recorded two successful transmission-experiments with *Ceratophyllus fasciatus* but it was later established that the fleas in question actually belonged to an allied species now called *Nosopsyllus punjabensis* However experiments with *N fasciatus* were made by McCoy[166]—who was able to transmit plague with these fleas from rats to ground squirrels—and a few years later by Bacot & Martin[8] Although chary of drawing stringent conclusions Bacot & Martin found that blockage of the proventriculus occurred more readily in X cheopis than in *N fasciatus* and gained in general the impression that the former could transmit plague more easily than the latter

In a series of further experiments Bacot[7] used batches of 100-300 starved *N fasciatus* which had been infected by feeding on moribund

plague-mice and were then kept in cages covered with wax cloth and stored at an average temperature of 7.5°C. By adding healthy mice to these cages from time to time, Bacot was able to prove that plague persisted in the fleas of three cages for 29, 34, and 47 days, respectively.

Comparing the vector capacity of *N. fasciatus* with that of *X. cheopis* through individual feeding tests, Eskey & Haas,[54] and Burroughs[5] obtained the following results:

	Eskey & Haas		Burroughs	
	X. cheopis	*N. fasciatus*	*X. cheopis*	*N. fasciatus*
Average extrinsic incubation period (days)	21	41	12.6	16.6
Length of survival after blockage (days)	2.8	4.3	4.4	4.0
Percentage of infected fleas becoming vectors	20.0	20.0	37.7	12.7
Ratio of transmissions to fleas studied	0.42	0.33	0.660	0.208

Although, according to these observations N. fasciatus is a fairly efficient vector, it is often maintained that it plays a far less important role in the transmission of plague under natural conditions than *X. cheopis*. Hirst,[10] *while concluding from observations made in Japan that an epizootic* might be continued by *C. [N.] fasciatus* in the winter months for some time after the *X. cheopis* population has declined, maintained that there was "no evidence as yet that *C. [N.] fasciatus* or *L. musculi* can either initiate a rat epizootic or maintain it for any length of time in the entire absence of *X. cheopis*."

Eskey[51, 53] laid stress upon the fact that the extrinsic incubation period was much longer in the case of *N. fasciatus* than in that of *X. cheopis*. In his opinion plague outbreaks in warm countries, where *X. cheopis* was the vector, ran a rapid course with a high incidence of human infections. *In colder climates where N. fasciatus preponderated,* the outbreaks were prolonged and the incidence of human plague was low. For instance in Seattle, Wash., rat plague smouldered for ten years and yet only three human cases were recorded. While assuming that *N. fasciatus* played an important part in carrying over plague, Eskey, like Hirst, disbelieved that this flea could initiate epizootics.

Mohr,[18] dealing with *N. fasciatus*, stated that:

"... there has been considerable speculation regarding this flea's role as a plague vector. To it was attributed the occurrence of plague among domestic rats at Tacoma in 1942 because it was present in great numbers and because no Oriental rat fleas were found on *the plague infected rats. The evidence is equally good that the fleas of* meadow mice and possibly white footed mice with which the Norway rats were associated caused a secondary epizootic among the rats. No plague was recovered from rats which were far removed from association with the wild species and its fleas."

These statements deserve great attention in connexion with the question of whether or not *N. fasciatus* played an important role in the transmission of the infection during the past plague outbreaks in northwestern Europe.

Since, as stated earlier, it is difficult to believe that *X. cheopis* was rampant there in the past, one is led to believe that *N. fasciatus* and possibly *P. irritans* also were responsible for the spread of the infection in the historic plague outbreaks of northwestern Europe.[17, 19] No doubt can exist that these pests abounded then, so that their large numbers might have compensated for any shortcomings in their vector capacity. Moreover, there can be little doubt that widespread outbreaks in northwestern Europe like the "Black Death" were due to an invasion of plague from areas in which efficient vector species were present. However, while these considerations speak against the supposed role of *X. cheopis* in the plague outbreaks in Europe in the past, one should beware of being dogmatic when dealing with a problem, for the solution of which one has to depend on surmise rather than on factual data.

Observations in the Nilgiris district of south India suggested that *N. nilgiriensis*, a flea infesting mainly *Bandicota bengalensis kok*, was associated with *X. cheopis* in the transmission of plague.[137] This was confirmed by George & Timothy,[0] who found that, out of three *N. nilgiriensis* which had been fed on an infected mouse, two showed numerous plague bacilli in their stomachs one week later. Although they did not perform transmission experiments, these workers believed that this flea was "at least a weak vector of plague among rodents". Apparently it did not bite man.

In the opinion of Macchiavello,[159, 18] *N. londiniensis*, which took the place of *X. cheopis* in the highlands of Ecuador, probably played a role in the transmission of plague but was apparently a poor vector.

Monopsyllus anisus and, to a lesser extent, *Paradoxopsyllus* (formerly called *Ceratophyllus*) *curvispinus* have been considered as vectors of the infection during past plague outbreaks in Japan.[135] It is undecided to what extent *M. anisus*, which largely replaces *N. fasciatus* in China, is participating in the transmission of the disease. Wu[31] pointed out that during the epizootics at Shanghai in 1910 and 1911 most rats succumbed between October and April when, by analogy with later surveys, *X. cheopis* was presumably scarce but *M. anisus* and *L. segnis* were prevalent.

Leptopsyllidae

General agreement exists that *L. segnis* plays an almost negligible role in the transmission of plague, both because it is a poor vector and because it attacks man with the greatest reluctance. Experimenting with 23 fleas of this species, Eskey & Haas[4] noted that five (18%) became plague-infected. Apparently, however, these fleas were incapable of transmitting the infection.

Devignat[41] inoculating guinea-pigs with pools of different flea species obtained some positive results with *L. aethiopica*. However, he did not ascribe importance to this flea, which infested commensal rats in only a few localities.

Paractenopsyllus kerguisteli is probably of local importance in the spread of plague in Madagascar [219]

Pygiopsyllidae

In Java, as noted earlier, *Stivalius cognatus* (formerly thought to be *Pygiopsylla ahalae*) was found capable of transmitting plague from rat to rat and of biting man [247]

George & Timothy [70] were likewise able to transmit the infection with the aid of *Stivalius* (probably a mixture of *St ahalae*, *St aporus*, and *St ferinus*) collected from *Bandicota bengalensis kok* in the Nilgiris district of south India

Synosternus pallidus

As noted earlier, *Synosternus pallidus* was found to be frequent in French West Africa mainly on the floors of houses but also to some extent on the commensal rodents Opinions regarding the role played by this flea in the spread of plague were divided Advier [1] found that

> *Synosternus pallidus* is therefore not the usual parasite of the animals which form the principal reservoir of the plague virus In consequence of this biological characteristic the fleas of this species have little opportunity to ingest plague bacilli and even if they do they do not seem very capable of transmitting the infection They do not therefore play an important role in the propagation of the disease " [e]

On the contrary, Kartman [1 6] concluded from circumstantial evidence that in addition to *X cheopis*, *S pallidus* probably played a role in the transmission of plague from the rats to man, and was also a vector of the infection from man to man He stated in this connexion that, while the *cheopis* index was comparatively low (0 96) *S pallidus* not only abounded on the floors of the huts but also infested the rats to some extent

Synopsyllus fonquerniei

As mentioned earlier, *S fonquerniei* which was formerly rare on the Madagascar high plateau has become abundant since 1931

Dealing with the possible role played by this flea in the transmission of plague Robic [217] pointed out that contrary to *X cheopis*, *S fonquerniei* mainly infested the rats living outdoors and was more frequent during the cold seasons *S fonquerniei* had been proved to be a vector as well as a carrier of *P pestis*, but since it always occurred in association with *X cheopis* it was difficult to assess its role in the spread of plague

Similarly it was stated by Girard [78] that since plague had spread before *S fonquerniei* had become abundant and since this flea was rarely found inside houses its importance as a vector was probably not considerable

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However, it might be capable of playing a role in the perpetuation of the infection among the outdoor rats which were more numerous and in closer contact with one another than those living indoors.

Wild rodent fleas

It is not possible to assess the role played by the wild rodent fleas in the transmission of plague in as systematic a manner as was done in the case of the commensal rodent fleas not only because, as shown by Annex I table V (see page 638) a great number of species are involved but also because their individual vector capacities have not been uniformly well determined.

Generally speaking it may be maintained that as in the case of the rat fleas marked differences in vector capacity exist between the various wild rodent fleas found in plague foci. A number of the species studied in the laboratory proved to be almost as capable of transmitting plague as *X. cheopis* others appeared to be mediocre or even poor vectors while some seemed to be incapable of conveying the infection. However while it seems permissible to use the positive findings so far obtained for an assessment of the comparative vector capacity of the various species one should be chary of concluding from laboratory observations made during limited periods and not infrequently with an inadequate number of fleas that a given species is actually unable to transmit plague. At least as has been shown by the observations of Golov & Ioff[84] practically all the flea species, regardless of whether or not they could act as vectors individually could convey the infection when attacking susceptible rodents en masse.

It is, however, interesting to note that in the opinion of Eskey[5] it is possible that more wild rodents contract plague from infected flea faeces cannibalism and by eating infected fleas than by the bites of fleas. In support of this contention Eskey stated that

> " the fact that it has been possible to obtain a large number of positive guinea pig inoculations from pooled specimens of fleas collected from several different rodent hosts which showed no evidence of being infected themselves yet which harbored infected fleas strongly suggests that these infected insects may feed on their hosts without infecting them "

In evaluating this statement, one should give consideration to what was said in chapter 5 with regard to the diagnostic value of tests using pools of organs or fleas. Although such tests are most accurate in determining the presence of *P pestis*, they do not permit of conclusions as to how numerous the bacilli were and what reactions they produced. Hence the tests to which Eskey referred merely indicated that some—or perhaps even only one—of the pooled fleas were infected. On the basis of this evidence one would hardly be justified in drawing conclusions about the ability of the fleas in question to transmit plague when they were infective.

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Generally speaking, it may be maintained that, as in the case of the rat fleas, marked differences in vector capacity exist between the various wild rodent fleas found in plague foci. A number of the species studied in the laboratory proved to be almost as capable of transmitting plague as *X. cheopis*, others appeared to be mediocre or even poor vectors, while some seemed to be incapable of conveying the infection. However, while it seems permissible to use the positive findings so far obtained for an assessment of the comparative vector capacity of the various species, one should be chary of concluding from laboratory observations, made during limited periods and not infrequently with an inadequate number of fleas, that a given species is actually unable to transmit plague. At least, as has been shown by the observations of Golov & Ioff,[34] practically all the flea species, regardless of whether or not they could act as vectors individually, could convey the infection when attacking susceptible rodents en masse.

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" . . . the fact that it has been possible to obtain a large number of positive guinea-pig inoculations from pooled specimens of fleas collected from several different rodent hosts which showed no evidence of being infected themselves, yet which harbored infected fleas, strongly suggests that these infected insects may feed on their hosts without infecting them."

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A much debated question is to what extent the wild rodent fleas are responsible for the transmission of plague to man. In some of the foci the wild rodents are hunted for the sake of their fur, meat, or fat and, even in other foci, a study of the case histories showed that some of the patients had had direct contact with diseased or dead rodents. The preponderance or frequency of axillary buboes noted in the initially affected individuals in some of the foci seemed to furnish proof that infection was often due to direct contact and not to the bites of infected fleas.

Observers of sylvatic plague in the USA, e.g., Meyer,[1, 17, 176] Eskey,[5] and Wheeler & Douglas[6, 7] who have recently dealt with this problem, shared the opinion that the wild rodent fleas were responsible only in part for the conveyance of the infection to man. Thus Eskey[5] estimated that out of approximately 40 cases of human plague of supposed wild rodent origin which had been recorded in the western States of the USA up to 1938 less than half had been caused by the bites of infected fleas.

Meyer[176] studying the histories of 50 bubonic plague patients in the western States of the USA whose infection appeared to have been of wild rodent origin, found that statements relative to the location of the buboes were available in 39 instances. Of these 39 patients 17 had primary buboes located in the groin, and 12 had primary buboes in the axilla. Meyer felt convinced that the infection of the patients with groin buboes was the result of flea bites on the lower limbs, but maintained, with much reason, that the patients with axillary buboes might have contracted infection when handling sick or diseased rodents or through the bite of infected animals.

As shown by the evidence discussed above, the possibility that man may contract infection by coming into direct contact with plague-affected wild rodents deserves serious attention, and all possible steps should be taken to avert this danger. At the same time, however, it is probable that at least half or probably even a majority of the patients who had contracted bubonic plague in the wild rodent foci had been infected through flea bites.

Ctenocephalides species

Both the common cat flea, *Ct. felis felis*, and the dog flea, *Ct. canis*, proved rather feeble plague vectors under experimental conditions. Twenty-seven transmission experiments made with *Ct. felis felis* by the Plague Research Commission[20] all gave negative results. Verjbitzki[64] obtained a few positive results when exposing healthy rats to the bites of groups of 10 cat fleas which had fed on infected animals 12-48 hours previously, and also when making identical tests with groups of 5-10 *Ct. canis*.

Eskey & Haas[54] reported that out of 28 cat fleas which had fed on a plague-affected guinea-pig two became infected. Apparently, however, it was not possible to test the vector capacity of these fleas.

Wheeler & Douglas[277] obtained positive results when (*a*) exposing a group of 50 cat fleas immediately after the death of their infected host

(a mouse) on a healthy guinea pig and (*b*) starving these fleas for two days after the death of the guinea pig and then again exposing them on a healthy guinea pig. However, these workers failed to obtain positive results when making individual transmission tests with *Ct. felis felis* even though 12 of the 14 fleas used were afterwards proved by histological examination to be plague infected. Wheeler & Douglas expressed the opinion that possibly "a single *C. felis* flea is not anatomically suited to regurgitate a sufficient number of *Past. pestis* to produce an infection". This possibility is supported somewhat by the fact that organisms were never found in the oesophagus of this species.

Occasional findings of naturally infected *Ct. canis* have been recorded, particularly by Blanc & Baltazard [9] who established through animal experiments the presence of *P. pestis* in 50 fleas of this species which had been collected from a plague infected dog. These workers were also successful when performing an animal experiment with three *Ct. felis felis* found on a plague infected cat. However, an inoculation test with 20 fleas of this species collected from a second plague infected cat which had been sacrificed gave a negative result. Hervaux & Toumanoff [83] failed to produce infection in experimental animals with both cat and dog fleas which had been collected in plague infected houses.

In view of this evidence one ought to share the disbelief of Hervaux & Toumanoff that the common cat and dog fleas play a role in the transmission of plague under natural conditions. However, as stated in chapter 6, both cats and dogs may prove dangerous by bringing infected rodent fleas into houses.

In a report on plague investigations in the Cumbum Valley of south India the finding of one infected *Ct. felis orientalis* in a plague infected house was recorded.[116] It was added that since this flea prevails largely on cows and buffaloes—particularly on the calves—and the farmers live in close association with their cattle, it might be potentially dangerous. Some transmission experiments were therefore attempted but did not give conclusive results.

Devignat [44, 45] making pooling tests with various flea species repeatedly obtained positive results with groups of *Ct. felis* [illegible] a flea replacing *P. irritans* as a human parasite in the Belgian Congo. However, many transmission experiments carried out with *Ct. f*[illegible] gave negative results so that its vector capacity appeared to be [illegible]

E[illegible] *gallinacea*

That *E*[illegible] may be capable of playing a r[illegible] in the t[illegible] of plague was shown by Wheeler et al. [illegible] when making pooling tests with 70 fleas of this species [illegible] from burrowing owl (Spe[illegible]) [illegible] ground squirrel of California

The importance of these findings was confirmed by Burroughs [5] who succeeded in transmitting plague not only with groups of *E gallinacea* but also with individual feeding tests As far as could be established, the vector capacity of this flea was higher than that of *N fasciatus*

Tunga penetrans

Devignat [44] [45] stated, without giving further details that he had obtained positive results when making pooling tests with *Sarcopsylla* (scilicet *Tunga*) *penetrans*

Pulex irritans

As shown by the summaries published by Wu in 1936 [81] and Girard [79] in 1943 as well as by a study of the recent literature, transmission experiments with *P irritans* have been made by several workers, e g, the Plague Research Commission, [92] Verjbitzki,[264] Long [151] Colichon Arbulu & Ramos Diaz (quoted by Ramos Diaz [211]), Blanc & Baltazard,[17] [0] and Burroughs [5] All these workers were successful only when using numerous or at least several, *P irritans* for their tests, the minimum number apparently being five fleas in one of the positive transmission experiments recorded by Blanc & Baltazard [0]

Burroughs [25] who seems to have been the only worker making individual feeding tests with *P irritans* was unable to transmit plague by means of this technique However he established the important fact that one of the 57 fleas of this species used for such tests became blocked on the 11th day after infection As it died one day later, its vector capacity could not be studied In this connexion Burroughs maintained that since his *P irritans* strain had lived on deer for generations better results might have been obtainable had a strain adapted to rodents been available Nevertheless his observation on the occurrence of blockage in this species leaves no room for doubt that *P irritans* is capable of transmitting plague through its bites

Findings of naturally infected *P irritans* in plague houses and on plague patients have been recorded on a few occasions Thus the Plague Research Commission [91] established the presence of *P pestis* in 1 out of 85 *P irritans* collected in plague houses—a result which stands in marked contrast to the 27 positive findings made in the case of 77 *X cheopis* which had been obtained simultaneously

Similarly, as quoted by Girard,[79] out of 142 *P irritans* collected during a plague outbreak at Yura Japan, in 1908 seven (4 9%) were found plague infected, as compared with 22 5% *X cheopis* 27% *N fasciatus* and 44 6% *L segnis*

Wu Lien teh & Pollitzer [83] were able to infect guinea pigs with groups of three and two *P irritans* respectively which had been collected during a plague outbreak in the Tung liao area of south Manchuria in 1928 It is

noteworthy however that this epidemic was of a quite unusually severe character. The group of three fleas—which in contrast to the other group produced acute plague in the guinea pig tested—had been collected in an inn used by the villagers as a "plague hospital" where during his initial survey Pollitzer found 12 dead bodies besides one patient who eventually recovered. Jettmar [129] working in the same village in the following year when the plague situation was less serious found no further evidence incriminating *P. irritans*.

Ramos Diaz [11] obtained a positive result with a pool of 45 *P. irritans* collected from the garments of a plague affected family at Lambayeque, Peru.

The findings of Blanc & Baltazard [20] in a plague area of Morocco may be summarized as follows:

	specimens collected	Number of pools tested	pools found plague-infected
P. irritans	1 772	53	25
X. cheopis	580	21	6
Pediculus h. humanus	2 882	42	31

Blanc & Baltazard succeeded in one of several attempts in infecting guinea pigs through the bites of numerous *P. irritans* collected in plague houses and also obtained positive results when inoculating guinea pigs with fleas of this species which had been collected in a plague free locality and then fed on moribund plague patients. Details of these experiments were as follows:

(1) Two hundred *P. irritans* were fed on a patient with bubonic plague $3\frac{1}{2}$, 2 and $\frac{1}{2}$ hours respectively before the death of the sufferer. Later these fleas were fed once daily for nine days on a convalescent and were then triturated and inoculated into a guinea pig which succumbed to plague in two days.

(2) Two hundred *P. irritans* were fed twice daily for four days on a plague patient with an axillary bubo who died soon after the last exposure of the fleas. On the following day the 55 fleas which had survived were used for an animal experiment, the guinea pig inoculated with their triturate succumbed to the infection after seven days.

The question of to what extent *P. irritans* is of actual importance in the transmission of plague has been the subject of much debate.

Plague workers—not only in India but also in the [illegible] to the east of that subcontinent and in Egypt—were mostly [illegible] to accept the views of the Plague Research Commission [illegible] summed up by Petrie [159] as follows:

* Bubonic plague [illegible] [illegible] experience and by the [illegible] [illegible] when they [illegible]

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P irritans	1 772	53	25
X cheopis	580	21	6
Pediculus h humanus	2 882	42	31

Blanc & Baltazard succeeded in one of several attempts in infecting guinea pigs through the bites of numerous *P irritans* collected in plague houses and also obtained positive results when inoculating guinea pigs with fleas of this species which had been collected in a plague free locality and then fed on moribund plague patients Details of these experiments were as follows

(1) Two hundred *P irritans* were fed on a patient with bubonic plague 3½, 2 and ½ hours respectively before the death of the sufferer Later these fleas were fed once daily for nine days on a convalescent and were then triturated and inoculated into a guinea pig which succumbed to plague in two days

(2) Two hundred *P irritans* were fed twice daily for four days on a plague patient with an axillary bubo who died soon after the last exposure of the fleas On the following day the 35 fleas which had survived were used for an animal experiment the guinea pig inoculated with their triturate succumbed to the infection after seven days

The question of to what extent *P irritans* is of actual importance in the transmission of plague has been the subject of much debate

Plague workers—not only in India but also in the plague areas to the east of that subcontinent and in Egypt—were mostly inclined to accept the views of the Plague Research Commission aptly summed up by Petrie[198] as follows

"Bubonic plague rarely spreads by direct contact a statement supported by hospital experience and by the circumstance that multiple cases in houses are infrequent and that when they do occur the attacks are often almost simultaneous as if they were due to a

common infecting agent The Commission taking these considerations into account and having regard to the slight septicaemia in human plague as compared with that in rats thought that transmission from man to man by the human flea was a rare occurrence "

Petrie added that observations on *P irritans* in Upper Egypt by Petrie & Todd [199] although showing a high infestation during the plague season, did not indicate that these fleas took any share in the spread of the infection

Further, should fleas or other human parasites play an important role in the spread of plague, one would find a considerable incidence of bubonic cases in outbreaks of pneumonic plague, a form of the disease often characterized by a particularly marked septicaemia Actually the incidence of bubonic plague in pneumonic epidemics running their course independently of rodent epizootics is almost negligible [98]

Nevertheless, as recorded by Girard,[9] observers in some plague areas, particularly in Morocco maintained that *P irritans* played an important role in the spread of human plague which, in these areas, often involved several members of a family rather than individuals The importance of this flea, and also of the human louse was particularly emphasized by Blanc & Baltazard [20] who, although admitting the role of the rodent fleas as initiators of all plague manifestations reached the conclusion that there were no epidemics or endemo epidemics of plague without interhuman transmission in which the principal role is played by human ectoparasites [f]

Scrutinizing the postulates of Blanc & Baltazard Girard [9] expressed the opinion that, in place of the classical assumption that plague invariably spread from the rodents to man and not in the opposite direction as well a new concept based on the assumption of a spread of the infection from rodent to man man to man, and back from man to the rodent ought to be adopted He emphasized, however, that at least as far as his many observations in Madagascar went free living *X cheopis* rather than *P irritans*, were responsible for the interhuman spread of plague and its retrocession from man to the rats

At the same time, Girard laid stress upon the fact that, while the formula ' rat man man rat comprised all epidemiological possibilities each link of this chain was apt to assume greater or lesser importance according to the conditions peculiar to each plague area Therefore, without refuting claims made in regard to a given area, one ought not to consider them generally valid

One must certainly agree with this conclusion It is conceivable that in areas like Morocco, where *P irritans* occurs abundantly it might play an important role in the transmission of plague, the high incidence of this species compensating for what it lacks in vector capacity However, it

[f] pas d ép démies ou d endémo-épidemies de peste sans transmiss on interhuma ne pour laquelle se placent au premier plan les ectoparas tes humains

is certain that in other plague areas, e.g. China, India and Madagascar, the role of this flea is negligible, the transmission of the infection depending upon the rat fleas, particularly X. *cheopis*.

Role of Rodent Fleas in Perpetuation of Plague

Rat fleas

As shown by the Plague Research Commission[202] and other early observers, infected rat fleas, if kept in the laboratory under suitable climatic conditions, could convey plague after fairly prolonged intervals—43 days after infection in the case of X. *cheopis* (Otten[193]) and 47 days after infection in the case of *N. fasciatus*.[7] Nevertheless, the concept that the rat fleas might form a link in the perpetuation of plague as well as serving as vectors of the infection received tardy recognition. In fact, evidence proving that fleas may act as "preservers" of plague was brought forward in the case of species infesting wild rodents long before analogous observations were made in the case of the rat fleas.

Studying the problem of plague recrudescence in the Cumbum Valley of south India, George & Webster[71] commenced their investigations by collecting "wandering" fleas (X. *cheopis* and X. *astia*) in houses or from rat burrows in houses where rat falls had been observed. These fleas were tested with the aid of culture methods or animal experiments. In 196 tests made in this manner, they found infected fleas in 41 instances:

Time interval after the last rat fall observed in the house	*Number of times plague was demonstrated in the trapped fleas*
20 days	33
3.5 weeks	7
13 weeks	1

* Pairs of "de-fleaed" guinea-pigs allowed to run loose overnight were used to collect rat fleas in the houses, while burrows were tested by leaving a de-fleaed rat tethered by a piece of wire 4-5 feet (1.2-1.5 m) long in the burrow overnight.

Further experiments were carried out at a mean temperature of 79°F (26°C) in specially constructed huts where (*a*) healthy rats were allowed to burrow, (*b*) large numbers of X. *cheopis* and X. *astia* were released, (*c*) infected rats were introduced, and (*d*) following the death of both the healthy and infected animals, normal rats were introduced after the fleas had been starved for varying lengths of time. It was thus proved that the fleas were capable of transmitting plague after periods of starvation lasting up to 29 days.

Commenting upon these findings, George & Webster pointed out that "whether such periods of starvation are usual in nature is unknown. It seems more probable that wild infected fleas obtain at least an occasional meal, in which case the length of life would presumably be longer and the occasional host would sometimes become infected."

Investigations by *Girard & Estrade*,[81] started simultaneously with and independently of, the work of George & Webster, and supplemented by the further exhaustive studies of Girard[74-76, 79] and Robic,[17] also demonstrated the importance of free living *X. cheopis* in the perpetuation of plague. These fleas, which found almost ideal conditions for existence in the rice debris accumulated in dark corners of the houses on the Madagascar plateau were proved to harbour *P. pestis* in the absence of manifest plague for periods of up to three months.

On the basis of observations made in Brazil, *Macchiavello*[156, 158] emphasized the important role of *X. cheopis* when inhabiting the rat nests. In his opinion these fleas remained infected after their hosts had succumbed to plague or had become immune and were apt to spread the infection to newly-bred rats or to adult animals which reoccupied the burrows in question. Macchiavello added in a later communication[161] that according to observations in Peru, *X. cheopis* could remain infected in empty rat burrows for periods of up to six months or even longer.

Wild rodent fleas

Golov & Ioff[83] kept three species of wild rodent fleas found in south east Russia (*Citellophilus tesquorum*, *N. setosa* and *Ctenophthalmus breviatus*) at temperatures corresponding to those of the rodent burrows in winter and established that these fleas remained active and able to feed on hibernating rodents. If infected, they were able to survive for periods of up to 206 days, the plague bacilli in their faeces and stomachs remaining virulent.

Making further exhaustive observations on these and some other flea species infesting the wild rodents of south east Russia, *Golov & Ioff*[84] established that these fleas, if given an infective meal, were able to harbour *P. pestis* for periods of up to 220 days, at temperatures ranging from 14°C to 27°C (57°F to 80°F), and up to 396 days at lower temperatures—0°C to 15°C (32°F to 59°F). If kept within the latter temperature range, the fleas remained capable of transmitting plague after periods of starvation up to 150 days.

Considering these observations Golov & Ioff felt certain that the wild rodent fleas of south east Russia were not merely plague vectors, but also played an important role as preservers of the infection.

Field investigations made simultaneously with the work of Golov & Ioff by Pirie[200] in South Africa proved that the fleas of non hibernating wild rodent species were capable of playing an analogous dual role, carrying over plague for periods of two to three months, probably even for four months.

The results obtained by Golov & Ioff were confirmed by several other observers in south east Russia.

Thus Tumanski & Poliak [6] collecting fleas from rodent nests five months after an epizootic had ceased found two *N setosa* and three *Ctenophthalmus pollex* infected with plague

In order to study this problem experimentally Evseeva & Firsov kept a large number of suslik (*Citellus*) fleas underground at a depth corresponding to that of the rodent burrows Recovering batches of these fleas at different intervals of time the two workers were able to demonstrate the presence of *P pestis* in 9 out of 135 fleas kept underground for 7 months and 11 days

Tiflov & Ioff, carrying out similar investigations found *N setosa* capable of harbouring plague bacilli for at least 180 days

Observations similar to those described above were made by Barrera [1] [13] in Mendoza Argentina Examining a large number of wild rodents as well as their fleas about three months after an epizootic he found no evidence of plague in the rodents but obtained a positive result when making animal experiments with flea pools

Observations in the western States of the USA also indicated that the wild rodent fleas were apt to play an important role in the perpetuation of plague Eskey [5] while noting that infected *X cheopis* survived an average of not more than 16 days with a maximum of 36 days found that infected wild rodent fleas could survive for considerably longer periods and because they were capable of eventually becoming blocked could thus be instrumental in carrying over the infection during the hibernation period of the rodents

As has been discussed previously this conclusion was confirmed by the observations of Eskey & Haas [4] on the varying length of the extrinsic incubation period in plague infected fleas

Experimenting with hibernating ground squirrels and their fleas (*D montanus*) Prince & Wayson [09] obtained the following remarkable results

1 Six hibernating *C richardsoni* were stored for four months at 40°F (4 5°C) in separate nests

(*a*) Two of these ground squirrels were inoculated with plague and each was seeded with 100 normal fleas One squirrel died of acute plague after two weeks while the second which continued to hibernate apparently recovered from the infection The 23 fleas which were recovered after the end of hibernation were not infected

(*b*) Two of the squirrels were each seeded with 100 plague infected *D montanus* One of the 14 fleas collected after the squirrels had awakened produced plague when injected after trituration into a white mouse The squirrels themselves did not contract plague

(*c*) Of the 100 fleas seeded on each of the two control squirrels 50 % were recovered after 4 months and were able to reproduce

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2 (*a*) Four *Citellus townsendi* survived four months in hibernation after intracutaneous plague infection One sickened on the seventh day after awakening and died one day later of acute plague The three remaining squirrels proved plague free Out of 200 normal fleas which had been placed on the squirrels at the time of their plague inoculation, 42 were recovered at the end of hibernation and proved plague free (*b*) Fifty infected *D montanus* were placed on each of four hibernating normal *C townsendi* and left on them for four months Of the 47 fleas which were recovered alive at the end of the hibernation period 4 were plague infected One of the infected fleas was placed on a white rat This animal sickened 21 days later and died of acute plague on the 23rd day Two of the remaining infected fleas died within four days after removal, having failed to feed The fourth flea fed on each of five white mice during its survival for 10 days but did not transmit the infection

These observations leave no doubt that plague may be carried over the hibernation period not only by the rodents themselves but also by their fleas

Conclusions

In the opinion of some writers great care ought to be exercised when making conclusions from observations made under artificial conditions upon the role actually played by the fleas in the perpetuation of plague Thus, Eskey & Haas [54] stated that

> under natural conditions the average length of life of fleas is probably rather short otherwise animal infestation would be excessive because of the rapidity with which these parasites multiply Therefore as the length of the extrinsic incubation is increased the spread of the infection is not only delayed but the chances of the plague infected fleas surviving the required time for their bites to become infectious are decreased according "

Similarly Meyer [176] stressed the necessity for further field observations so as to ascertain the actual number of infected fleas which survive and act as preservers of plague because

> irrespective of the remarkable longevity of fleas under well chosen laboratory conditions which prevent the loss of water by the insects it is frequently overlooked that the mortality among vectors in abandoned burrows and nests is great and the life span correspondingly is short

It has to be kept in mind however that free living fleas which have opportunities for subsisting in a suitable environment like rice debris as well as the fleas which live in inhabited nests with a suitable microclimate lead a rather sheltered existence and therefore have good chances for prolonged survival There can be little doubt therefore that such fleas do play an important role in the perpetuation of plague

Role of Flea Transport in Spread of Plague

Although strictly speaking the object of the present discussion is to determine to what extent passively transported fleas are apt to spread plague it seems appropriate to introduce this subject by a short considera tion of the movements and transport of fleas in general

Widespread agreement exists that while the rodent fleas are prone to leave not only their dead hosts but also deserted burrows their active movements are restricted to a rather limited range However their jumping ability is remarkable

Experiments undertaken in this respect by the Plague Research Com mission [6] showed that guinea pigs which were allowed to run about on the floor of a flea infested warehouse or which were suspended in cages 2 inches (5 cm) above the floor all contracted plague whereas another group of these animals suspended in a cage 2 feet (61 cm) from the floor remained healthy because they did not become flea infested

Further observations have shown that in general fleas are capable of making vertical jumps of about 4 inches (10 cm) when starved and about 3 inches (7 5 cm) when gorged They can walk up a vertical sheet of glass for about 8 inches (20 cm) The distance they can cover by horizontal jumps is as a rule less than 6 inches (15 cm) [2 0 5] However *P irritans* can jump much higher and further—according to Mitzmain [181] 7 3/4 inches (19 5 cm) vertically and 13 inches (33 cm) horizontally

Burroughs found *P irritans* capable of jumping out of a 12 gallon (55 litre) earthenware crock which had been filled to within 13 inches (33 cm) of the top with dirt He added that *E gallinacea* although not as proficient as *P irritans* was also a good jumper while most of the wild rodent fleas studied by him were poor jumpers so that they could be retained safely in an enamel pan about 4 inches (10 cm) high

Fleas can be passively transported by (*a*) their hosts (*b*) transport vehicles (*c*) humans (on the persons or in the baggage of travellers) and (*d*) the movement of goods

The orbit of the passive transport of fleas on their specific hosts or on temporary hosts such as beasts and birds of prey is determined by the range of movements of these animals—a problem which received attention in chapter 6

The problem of the conveyance of fleas which have sheltered in transport vehicles needs no special attention because such insects are not likely to remain loose for prolonged periods but will seek a host or hide in goods Nevertheless such fleas may prove dangerous in places where plague patients have the possibility of using public conveyances for local transport

A case in point was observed during the 1920-1 pneumonic outbreak at Harbin where plague sufferers were quite frequently transported in rickshas The patient in question a Russian woman had had no contact with plague

cases but was certain that she had been bitten by an insect on the right leg while riding in a ricksha A few days later she developed a right femoral bubo Since rat plague was altogether absent there can be little doubt that she was infected through the bite of a human parasite which having left a plague patient, was lurking in the ricksha

The problems of a passive transport of plague infected fleas on the persons, and in the baggage of travellers, and in goods, deserve serious attention

Transport by travellers and their baggage

Considering the manner in which plague is spread at distance, the Plague Research Commission [70] laid stress on the results of experiments carried out with bundles of clothing and bedding collected from houses where plague cases had occurred With the aid of guinea pigs which were let loose on the opened bundles in a flea proof warehouse, two rat fleas and one human flea were recovered More important still, 1 of the 26 guinea pigs successively used for these tests contracted plague while control experiments with flea free bedding from a plague hospital gave negative results

The Commission assumed therefore, that the spread of the infection at distance was effected by infected fleas carried either on the person or in the bedding of travellers who had lived in plague houses or had visited them In the opinion of the Commission such fleas were not directly responsible for the causation of human plague (even the travellers carrying flea infested baggage were able to escape infection) but produced rat plague in the places to which they were transported

While it is generally admitted that plague infected fleas may be carried on the persons or in the baggage, of travellers, opinions vary considerably as to the frequency and consequently, the epidemiological importance of such a transport

Petrie & Todd [199] who made investigations in Upper Egypt similar to those of the Plague Research Commission in India, accepted the view of the Commission Observers in Madagascar [76, 79] also ascribed importance to a transportation of infected *X cheopis* by travellers, while Long [151] maintained that a conveyance of fleas on the persons or in the effects, of mule drivers was of importance in the spread of plague in South America

Hirst [103] while admitting that human migration may be a factor in the spread of plague postulated that it is comparatively seldom of primary importance '

Observers in Java were even more inclined to doubt the importance of a transportation of plague infected fleas by travellers Swellengrebel [n] found only two *X cheopis* and one *Stivalius cognatus* (*Pygiopsylla ahalae*) among the ectoparasites collected from the clothing or baggage of 56 790 travellers who had left the partly plague affected province of Malang Even

the clothes of a group of 1 829 persons, among whom there were 393 plague patients yielded only seven X cheopis In the opinion of Swellengrebel, the transport of fleas by travellers was therefore, a minor factor in the spread of plague in Java

Otten [19] incriminated the flea transport in the produce carried by villagers to the markets rather than travellers in general whereas Flu [6] postulated that the carriage of fleas in rice consignments was responsible for the spread of plague in Java

Observations by Pollitzer showed that rice transport played the principal role in the dissemination of plague in central and south China An occasional spread of the infection by travellers was noted but as was maintained by Sorel [39] in the case of Madagascar, such an importation of plague did not as a rule lead to the establishment of foci

All in all one ought to agree with the opinion of Hirst [103] that in general a spread of flea borne plague through travellers is of comparatively limited importance

Transport in goods

Most observers [*] are agreed that the transport of infected fleas in goods, particularly in grain raw cotton gunny bags rags and hides is of great importance in the spread of plague However the question of how far the infection can be carried by such fleas has been the subject of much debate

Hirst [103] whose investigations have done much to elucidate this problem maintained that

> " the available evidence indicates that when an overseas source of infection is but a few days removed an infected flea may be readily transferred directly in grain from the port of origin to the port of entry Otherwise it may be inferred that a plague epizootic has occurred among the rats on ship board The link between the ship epizootic and the shore rats or the rats of the lighters into which cargo is loaded or unloaded may be a plague rat but is much more likely to be a plague flea

Referring particularly to the importation of plague from Rangoon into the port of Colombo 1 234 miles (1 985 km) away Hirst stated that the transference of infected fleas all the way from Rangoon to Colombo without their feeding on ship rats *en route* is theoretically possible but the sea voyage is about two days too long to favour this mode of spread

In marked contrast to these statements it was claimed by Long & Mostajo [15] in 1934 and recently by Macchiavello & Mostajo Patron [160] that fleas in baled jute bags from Calcutta were responsible for the reimportation of plague into Peru

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[*] Campbell and Hopkins maintained that the trade in cotton found by King & Pandit to be of *paramount* importance in the dispersal of *X cheopis* and consequently in the spread of plague in south India did not play a role in East Africa The same observation was made by Roberts in regard to the transport of maize in Kenya

The statements made by Long & Mostajo were subjected to a painstaking inquiry by de Vogel,[266] who came to the following conclusions

(1) As far as could be established rat plague had been absent on the two steamers which had brought the suspected jute bag cargoes

(2) A study of the temperatures to which these cargoes had been subjected in transit rendered *it altogether unlikely that the fleas supposed* to have been enclosed in the jute bag bales at Calcutta could have survived or could have remained infective, throughout the journey to Peru

(3) It was striking that all the plague manifestations supposed to stand in causal connexion with the arrival of the jute bags had been observed in the zone of Peru where outbreaks of the disease used to be frequent, whereas the importation of such consignments into three ports situated outside this zone was not followed by an appearance of plague

De Vogel felt certain, therefore that the evidence brought forward by Long & Mostajo was not convincing enough to justify international action in regard to the jute-bag traffic

The validity of de Vogel s conclusions was contested by Macchiavello & Mostajo Patron [160] who pointed out, in particular that the plague manifestations suspected of having been caused by infected fleas enclosed in the jute bag bales had taken place during a season when there was usually no plague in Peru

They also maintained that

> facts observed and described in the past for which satisfactory explanations were not found could have been explained in the light of the hypothesis of Long & Mostajo For example the events that occurred in 1904 in Antofagasta Chile when the steamer 'Gladstone imported bales of jute bags and there sickened and died certain of the employees charged with the duty of examining the cargo the finding in 1929 in the same port of a certain number of fleas identified as *X. astia*, a flea native to India the only fleas of this species ever found in the Americas the occurrence of plague in 1930 after it had been absent for five years, these various incidents could be explained when official documents showed on each occasion that there had been imported some millions of kilograms of jute bags sufficiently in advance of the outbreak to account in a simple and complete form for the facts mentioned

Macchiavello & Mostajo Patron further claimed that ' the reappearance of plague in Peru, in 1944 and 1945, in the rural districts of Chiclayo even though the increase was not related to circumstances similar to the case of the 'Solafric revived suspicions of another importation of plague from India due to the active commerce in jute bags which has been maintained despite the war "

Macchiavello & Mostajo Patron stated that the suspected shipment of 400 bales of jute consisting of 120,000 bags, had reached the Chiclayo district under conditions which excluded local contamination or infestation with fleas Likewise, it could be shown that there had been no plague in the locality concerned for a number of years

Examining ten of the bales the two workers found 20 *P irritans* in the external wrappings and seven specimens of *Xenopsylla* two of which were alive in the interior of one of the bales All the *Xenopsylla* were in a fasting condition Inoculation of a triturate of the *Xenopsylla* into guinea pigs gave after four passages in series in these animals a picture of attenuated plague a result which in the opinion of Macchiavello & Mostajo Patron, was compatible with a long persistence of the infection in these insects

The two workers also had the opportunity of examining a consignment of 5,400 jute bags which had been landed in the port of Huacho They stated that during these investigations they were able to collect 24 fleas 10 of which were *X cheopis* 8 being alive Of these 8 fleas 6 produced plague in a guinea pig upon which they were allowed to feed Animal experiments with triturated fleas also gave positive results

Macchiavello & Mostajo Patron concluded that

" certain bacteriological peculiarities of the strains of germs the knowledge we have that plague exists in Calcutta the conditions of humidity and temperature in the holds of the vessels that arrive via the Straits of Magellan the insanitary conditions that the war has produced in India and which certainly had some effect on the measure of disinsectization which at one time were taken with cargos of jute bags the epidemiology of certain out of season outbreaks of plague that now as formerly are again making their appearance contrary to the annual cycle of plague in Peru and above all the findings reported herein do not leave the least doubt but that the hypothesis enunciated by Long & Mostajo more than ten years ago is correct

While no doubt can exist that the jute bag consignments examined by Macchiavello & Mostajo Patron contained infected and also in one instance infective *X cheopis* it is not easy to share their belief that these fleas had come from Calcutta where as shown by the following figures autochtonous plague was absent during the period 1944 6

Plague cases and deaths in Calcutta and in Bengal Province during 1944-6

Year	*Calcutta*		*Bengal*	
	cases	*deaths*	*cases*	*deaths*
1944		3*		3
1945		2*		0
1946		8*		3**

* Imported cases
** West Bengal only

However even if one could accept the statements of the above mentioned workers at their face value it is not likely that a long distance transportation of infected fleas plays a generally important role in the dissemination of plague For, if this were the case instances of a ship borne transference of this disease would have remained frequent in spite of the measures now universally adopted to reduce or even to abolish the rat infestation of sea going vessels Actually instances of a long distance spread of plague through the maritime traffic have almost ceased to occur There can be no

doubt, therefore, that infected rats rather than infected fleas, carried in consignments of goods are responsible for this dissemination of the infection

It is gratifying to note that large scale studies recently undertaken in the USA, fully support this contention

In order to assess the potential danger of an importation of plague into the USA through the very considerable jute imports from India (amounting to approximately 350,000 tons annually), Norris et al[187] recently resorted to three methods of investigation, namely

(1) A search for fleas in random samples of jute materials reaching the ports of San Francisco, California, and San Yuan, P R respectively

Painstaking examinations of (i) 880 random bolts of Hessian cloth, aggregating 152,000 yards, removed from 179 random bales, and (ii) wrappers of 67 gunny bag bales as well as 4 994 bags did not lead to the detection of even a single flea

(2) Test transports of *X cheopis* in the holds of vessels going from San Francisco to Hawaii or other Pacific ports and back which gave the following results

(*a*) Two cotton bags each containing 30 *X cheopis*, were placed at different levels in a jute bale, which was then wrapped pressed sewed and bound with metal strips and afterwards sent in the cargo hold of a vessel on a journey to Okinawa and back Upon return of the bale to San Francisco after 49 days, 57 of the fleas were found dead It was presumed that the three missing fleas had escaped before the cotton bags had been tied prior to shipment

(*b*) Two batches of *Y cheopis* (150 and 100 specimens respectively) were sent in wooden boxes (one of which contained pieces of freshly cut apples to supply moisture) in the holds of ships to the Hawaiian Islands and back When the ships returned to San Francisco after round trips lasting a little more than three weeks only, all the 246 fleas recovered were found to be dead Their microscopic examination did not reveal signs of starvation

(3) To gauge the effect of pressure as used in preparing jute materials for export batches of 25 *X cheopis* were placed at different levels inside bales of jute wrappers and these were then compressed at about 8,000 pounds (i e a pressure lower than that actually used for baling Hessian cloth) At the end of 10 minutes following pressure application, about one third of the fleas were found to be dead, at the end of one hour 50% were dead, and at the end of 72 hours, 75% Norris and his colleagues concluded therefore, that the pressure exerted in the baling process is an important factor in reducing chances of survival of fleas trapped within bales of jute products'

Considering the results of these investigations and bearing in mind that fleas in jute bales shipped from India would be subjected during the first

part of their journey to even more unfavourable climatic conditions than those prevailing in the holds of ships plying on the Pacific, one may safely state that the danger of a long distance transport in jute bales of plague infected fleas (which are more vulnerable than the normal fleas used in the above experiments) is rather remote or presumably even non existent.[h]

OTHER INSECTS

Human lice

Attention to a possible role of human lice (*Pediculus humanus capitis*) seems to have been paid first by the German Plague Commission,[71a] who noted a prevalence of cervical buboes in children infested with head lice and therefore suffering from eczema of the scalp.

Head lice were also suspected by Herzog (quoted by Dieudonné & Otto[46]) who was able to cultivate *P. pestis* from lice which had been collected from a moribund patient with a cervical bubo, and by de Raadt[210] who succeeded in infecting experimental animals cutaneously or subcutaneously with triturates of head lice found on a plague patient and on the dead bodies of plague victims.

Identical results were recorded later by Mostajo & Colichon Arbulu[183] who worked with nine *P. humanus capitis* collected from the dead body of a plague victim. These workers pointed out that the habit of the Indians in Peru of crushing lice or fleas with their teeth might be responsible for the occurrence of cases of angina pestosa (tonsillar plague).

Long[151] referring to the findings made by Mostajo & Colichon Arbulu stated that positive results were also obtained when a guinea pig was inoculated with the triturate of lice collected from healthy persons and then allowed to feed on plague infected guinea pigs. However, it was not possible to transmit the infection through live lice.

Further instances of infection in head lice collected from plague victims have recently been recorded in Madagascar[59] and in South Africa.[10]

Positive experimental results with *P. humanus* var. *corporis* (often wrongly called *P. vestimentorum*) have been recorded by Swellengrebel & Otten[247] who were able to produce plague in 7 out of 9 guinea pigs inoculated with batches of 11 250 such lice collected from the garments of plague victims. An identical result was also obtained by Sukneff.[11]

Tsurumi et al.[61] working with the same subspecies of louse were successful after many failures in transmitting the disease to a guinea pig on which plague infected specimens of these parasites had been exposed. Blanc & Baltazard[0] obtained the same result in one of ten attempts to transmit the infection to guinea pigs with the aid of live body lice found in a plague focus in Morocco. The animal in question succumbed seven

[h] The problem of flea-borne plague will receive further attention in a future chapter in which the ecology and epidemiology of the disease will be discussed.

days after it had received about 60 bites from body lice collected from three plague patients and afterwards fed on a convalescent

Blanc & Baltazard [20] also recorded the following observations on *P humanus* var *corporis*

(1) Positive results were obtained in guinea pigs which had been inoculated with triturates of 8-300 lice collected in plague infected hou s the experimental animals succumbing after 2 to 8 days, i e, more rapidly than those inoculated with triturated *X cheopis* or *P irritans*

(2) *P pestis* was found preserved in naturally infected lice for at least 12 days after the last infective meal

(3) The faeces of naturally infected lice usually contained virulent plague bacilli for periods of up to nine days

(4) Virulent plague bacilli were found in the carcasses of naturally infected lice for periods of up to 11 days

(5) It was possible to produce plague in 1 of 3 white mice into the nares or mouths of which a few drops of a suspension of infected triturated lice and their faeces had been instilled The mouse in question, which had been infected intranasally died after three days, and showed at autopsy cervical and mediastinal buboes, as well as congestion of a part of the left lung

As has been noted earlier Blanc & Baltazard postulated that human fleas and lice played an indispensable role in the epidemic spread of plague being mainly, if not solely, responsible for the infection of man It was found impossible, however, to consider their conclusion as generally valid because as has been shown by observations in most plague areas including those in China India and Madagascar, human vermin played but a minor role in the spread of the infection At the same time, however, it was admitted that, in some of the areas where it is abundant *P irritans* might be of importance in the transmission of plague to man, and this may also be said to hold true of the human louse mass attacks of which have been found capable of conveying the infection even to experimental animals

In chapter 1 attention was drawn to the statements of some observers who postulated that human ectoparasites were solely responsible for the spread of plague during the pandemic taking place at the time of Justinian Since according to a recently published note by MacArthur [15] rats were probably not absent from Europe at that time, the validity of these claims has become questionable Little doubt can exist however that, not only during that pandemic but also during the historic outbreaks in northwestern Europe in general, human parasites played an important adjuvant role in the spread of bubonic plague because they were then more abundant

Bed bugs (Cimex lectularius)

Evidence incriminating the bed bug as a potential or actual vector of plague has been adduced by a considerable number of observers

In 1897 Iamagiva[114] referred to a plague patient with a left femoral bubo who had apparently been infected by a bed bug because *P pestis* could be demonstrated in a swelling on the left knee of the sufferer which had been caused by a bug bite A similar, but less convincing observation made by Calmette & Salimbeni[31] has been recorded in full by Simpson[3]

Persistence of virulent plague bacilli in bed bugs collected in plague infected houses or infected in the laboratory, has been repeatedly observed first, by Ogata[190] and Nuttall[189] who noted that the organisms could survive in bed bugs kept at 68°F (20°C) for 72 hours but not for 120 hours Hunter[113] frequently found *P pestis* in bed bugs collected from plague infected houses or the bedding of patients

Making a thorough study of plague in *C lectularius* Verjbitzki[64] found virulent plague bacilli persisting for up to eight days in bed bugs which had been starved for 4 4½ months before being given an infective meal Bed bugs which had been starved for shorter periods remained infected less long However *P pestis* was present for three days in the faeces of such insects which had been starved for one month before infection

Jordanski & Klodnitski[134] found that the number of plague bacilli in the stomachs of bed bugs increased from the third to the sixth day after they had been given an infective meal Involution forms appeared later and the micro organisms eventually became invisible Nevertheless it was possible to obtain positive cultures from plague infected bed bugs up to 35 days The two workers recorded in a second paper[138a] that 2 out of 13 bed bugs fed on a plague infected mouse survived for 83 days *P pestis* could then be demonstrated in these insects five days after they had been fed on a healthy animal

In contrast to Verjbitzki Bacot[7] found that for some *C lectularius* and probably for all newly hatched ones a meal of septicaemic blood from a mouse dying of plague was fatal but that those which were not killed by the infecting meal were capable of carrying *P pestis* for quite prolonged periods Involution forms were possibly still present in one bed bug kept at 80°F (27°C) for 60 days after the infective meal

Cornwall & Menon[38] infecting bed bugs either on guinea pigs or by feeding them through a membrane of rabbit skin on citrated rabbit blood mixed with *P pestis* also found that following an infective meal a large proportion of these insects succumbed within a few days In some however the bacilli multiplied and could remain alive and virulent for periods of up to 38 days

Positive results were obtained by Wu Lien teh & Pollitzer[33] when inoculating a guinea pig with the triturate of three bed bugs found in an inn which had served as a temporary plague hospital

Working with bed bugs which, after a period of starvation ha permitted to feed on plague infected rats or mice, Novikova & Lalaz demonstrated the presence of *P pestis* in the faeces of 37% of these the number of the organisms becoming maximal 5 12 days after the i meal As shown by the cultivation of bed bugs which had been tri and suspended in saline, the infection persisted in them for up to 14

Besides demonstrating that *P pestis* could persist in *C lect* Verjbitzki [264] and Bacot [7] were able to transmit plague to health ratory animals through the bites of infected bed-bugs

Permitting his infected bed bugs to feed on the shaved abdom 41 guinea pigs, Verjbitzki was able to transmit plague six times but seven successes in 25 guinea pigs when he exposed the bed bugs shaved ears of the animals Verjbitzki established that the minimum n of bed bugs required to produce plague in a healthy animal through was three, and that no conveyance of the infection took place mor five days after the infection of the bed bugs

Bacot [7] stated that, in his experience, 'only on two occasions did follow from the bite of infected bugs and there was one doubtful c infection when a rat fell sick and showed some of the symptoms animal suffering from pest, but it subsequently recovered

As can be gathered from Bacot's records one of the animals in fatal plague resulted was a rat, and the other, a mouse He added th mouse had been put into a glass jar together with five bed bugs whic survived for 48 days after infection On the following morning on of these insects could be recovered, the others having probably been by the mouse After five days the mouse succumbed to typical plagu involvement of the lymph nodes in the right groin and both axillae

Successful transmission experiments were made also by Wall Novikova & Lalazarov [188] and Balfour (quoted by Dieudonne & Ot Walker was able to produce plague in a rat by exposing it to the bi bed bugs which had fed on a plague patient Novikova & Lala succeeded in transmitting plague to a mouse through the bites of a be which had been infected in the laboratory According to Dieudon Otto Balfour was able to transmit plague from infected to healthy gu pigs through the agency of bed bugs

As summarized by Dieudonne & Otto,[48] and by Wu, [81] some obse postulated on epidemiological grounds that bed bugs played an impo role in the natural transmission of plague Walker [263] in particular ins that these parasites had been responsible for the spread of the infe during an outbreak in Burma because they had been plentiful at the whereas fleas appeared to be absent Similarly, Fox [67] postulated that bugs had been responsible for the spread of the infection during a s outbreak of plague at Iloilo in the Philippines where no evidence o epizootic could be found However, when it is considered how dif

it sometimes is to find plague infected rats or their fleas during an outbreak, little credence can be given to these and similar contentions

It is significant that the workers who studied the problem of plague in the bed bugs thoroughly were rather averse to the idea that these parasites played a dangerous role

Verjbitzki [64] maintained in this connexion that bugs which have sucked their full complement of blood do not as a rule bite again for a considerable interval

He showed however that bed bugs which had been interrupted during the act of feeding were inclined to feed again and were thus able to convey infection from an infected to a healthy host He also found that the crushing of infected bugs *in situ* during the process of biting occasioned in the majority of cases the infection of the healthy animal with plague

Bacot [7] while in accord with Verjbitzki s findings pointed out that

(1) A transmission of plague through the faeces of infected bugs could be ruled out because these insects do not defaecate during the act of feeding and hurry after their meal to some nook or cranny to digest at leisure

(2) The development of *B* [*P*] *pestis* within the crop of bugs differs generally from that which takes place in the stomach of the flea in respect of its slower and looser growth this limitation of activity being accompanied by and possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop

(3) " The absence of any definite valve between the pump and the crop together with the looser nature of the growth within the bug preclude the idea of such mechanical blockage as causes regurgitation and mouth infection by fleas

Cornwall & Menon [38] were also convinced that plague infected bed bugs could not regurgitate their stomach contents during the act of feeding They were able to find plague bacilli in the proboscides of bed bugs up to 46 hours after feeding and therefore considered it possible that infection of a healthy host might result from the washing out of these bacilli from the proboscis of a bed bug whose feeding on an infected host had been interrupted Nevertheless Cornwall & Menon maintained that plague was not likely to result from the bites of infected bed bugs

While this contention deserves attention it cannot be denied that bed bugs are capable of transmitting plague However there is no reason to assume that they play more than an occasional or at most an adjuvant role in the conveyance of the infection

Lice of rodents and other animals

As was established by Simond [33] and confirmed by many subsequent workers the lice of plague rats are apt to harbour *P pestis* Identical observations were made in the case of *Neohaematopinus columbianus*—the louse

of the Californian ground squirrel—by McCoy,[160] who found that these parasites remained infective for two weeks and were capable of conveying plague to experimental animals

The presence of *P pestis* in a louse occurring on tarabagans (*Marmota bobac*) and identified as *Neohaematopinus citelli* (' *Linognathoides*' *citelli*) was demonstrated by Sukneff,[241] who obtained positive results when inoculating guinea pigs with triturates of such lice collected from either naturally affected or artificially infected Siberian marmots [4]

A thorough study of the tarabagan louse was later made by Jettmar [127] He established that *P pestis* rapidly multiplied in the stomachs of these lice which invariably died 2 3 days after an infective meal Plague bacilli were also plentiful in the faeces of the infected lice and persisted in their carcasses for about two weeks Exposure of 40 live specimens of this louse which had been collected from an artificially infected tarabagan produced acute plague in a sisel (*Citellus undulatus*) The tarabagan louse was found capable of biting man but was apparently unable to survive long when separated from its specific host

These findings deserve attention because there can be no doubt that the rodent lice play an adjuvant role in the transmission of plague from animal to animal

Blanc & Baltazard [20] were able to demonstrate the presence of virulent plague bacilli in *Pedicinus albidus* which had been collected from artificially infected specimens of its specific host—the Barbary ape *Macaca sylvanus* They found the louse of the pig (*Haematopinus suis*) not only capable of harbouring *P pestis* but also of conveying the infection to guinea pigs

Mites

It was maintained by Russo [227, 23] that free living mites of the family of Tyroglyphidae, which were apt to feed on the carcasses of plague affected rodents could harbour and transmit *P pestis* He incriminated, in particular, *Glyciphagus domesticus* and *Tyroglyphus siro*

Yamada (quoted by Mitsuhori [180]) established that *Liponyssus nagoyoi* (family of Dermanyssidae) a parasite of the commensal rats—particularly of *R rattus*—in Japan, could transmit plague from animal to animal As Mitsuhori added this mite which was possibly identical with *Lyponyssus bacoti* could attack man

According to a statement made by Jorge,[135] Bacot & Stanley Hirst succeeded in conveying plague from rat to rat with *Laelaps echidninus*, a mite infesting *R norvegicus* in many parts of the world

Ticks

Paying attention to the ticks infesting rats suffering from plague', Skinner [236] was able to prove that these parasites could harbour *P pestis*

Findings made in the case of other tick species may be summarized as follows

Marmot ticks Skorodumoff [3] was able to prove with the aid of animal experiments that ticks (? *Rhipicephalus haemaphysaloides*) collected from naturally plague affected tarabagans (Siberian marmots) contained virulent *P pestis* Identical findings were made by Tikhomirova & Nikanoroff and by Borzenkov & Donskov [1] in the case of *Ixodes autumnalis* a tick infesting the marmots of south east Russia

Suslik ticks As summarized by Wu [81] the suslik (*Citellus*) ticks were incriminated by several plague workers in south east Russia particularly by Tikhomirova & Nikanoroff [5] who proved the presence of *P pestis* in *Rhipicephalus schulzei* with the aid of culture tests and animal experiments, and by Golov & Kniazevski [8] who established that the larvae and nymphs as well as the adults of this tick could harbour the infection

Argas persicus Faddeeva [58] proved by cultivation and animal experiments that *Argas persicus* (a free living tick) when fed upon guinea pigs in the septicaemic stage of plague could remain infected for periods of up to 110 days

Dermacentor silvarum Sassuchin demonstrated that virulent plague bacilli could persist for up to 35 days in the nymphs of *Dermacentor silvarum* which during its larval and nymph stages infested susliks or other wild rodents and when adult fed on camels horses or man As was afterwards shown by Sassuchin & Tikhomirova [6] the larvae and nymphs but not the adults of this tick could harbour *P pestis* even though over 60% of them died when given an infective meal The organisms persisted in the surviving larvae for 2 10 days and in the nymphs for 2 6 days but invariably disappeared during the moulting stages

Hyalomma volgense Borzenkov & Donskov [1] proved that the larvae and nymphs as well as the adults of the cattle tick *Hyalomma volgense* were apt to harbour *P pestis* in their gastro intestinal tracts after they had been fed on plague infected guinea pigs jerboas or marmots the organisms remaining viable in the larvae for seven days in the nymphs for three days and in the adults for at least 11 days Borzenkov & Donskov succeeded in conveying plague to healthy rodents through the bites of these ticks and also demonstrated the presence of virulent *P pestis* in the faeces of infected specimens

Rhipicephalus sanguineus Blanc & Baltazard [0] summarizing observations they had made of *Rhipicephalus sanguineus* a tick principally infesting dogs stated that under the conditions of our experiments it did not transmit plague mechanically when taking its first meal on an infected guinea pig and continuing to feed after several days on a healthy guinea pig

Since, on the other hand, the plague bacilli could not resist the profound changes taking place during the moulting stages of these ticks one can conclude that, as far as the available evidence goes, *Rhipicephalus sanguineus does not seem to play any role in the natural transmission and preservation of plague* [1]

Opinions regarding the role played by the ticks in the transmission of plague were sharply divided Sassuchin & Tikhomirova [-6] like Blanc & Baltazard, came to the conclusion that the species they worked with were of no importance in this respect On the other hand as summarized by Wu [81] Tikhomirova & Nikanoroff [957] asserted that the suslik ticks particularly *Rhipicephalus schulzei* on account of their great prevalence eagerness to change hosts, capacity for transmitting and preserving other infections, blood sucking faculty and hardiness, might, under equal conditions prove more efficient vectors than other insects, e g fleas and lice and might on account of their aptitude for changing hosts be responsible for an extension of the enzootic foci

In evaluating these divergent opinions, one is led to believe that different tick species might play different roles in plague so that no hard and fast general rule can be laid down However while admitting that species like the suslik ticks are of some importance, one cannot share the opinion of Tikhomirova & Nikanoroff and some other workers in south east Russia that the ticks play not an adjuvant, but the principal, role in the transmission of plague No other arthropod vector surpasses or even equals the rodent fleas in this respect

Flies

Observations on the presence of *P pestis* on or in houseflies (*Musca domestica*) were made by some early workers, e g Yersin [85, 286] Albrecht & Ghon," and Nuttall [189] While Albrecht & Ghon merely established that flies which had come in touch with plague material were apt to become externally contaminated, Yersin and Nuttall found that *P pestis* was present in the gastro intestinal tracts and faeces of these insects

Nuttall came to the conclusion that plague infection was fatal for the flies, death occurring more rapidly the higher the temperature rose e g after 3 days at 23° 31°C This opinion was also held by Gosio [86] who further claimed that fly larvae which had been fed on plague organs could develop normally while continuing to harbour *P pestis* but that the adult flies developing from them succumbed to the infection in 15 24 hours However, Hunter [113] and Jettmar [1 8] were unable to confirm that plague infected houseflies showed any unusual mortality

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l n a pas dans les conditions de nos expériences transmis la peste mécaniquement en prenant la première moitié de son repas sur cobaye infecté et en l achevant en plusieurs jours sur cobaye neuf Comme d autre part le virus ne résiste pas à l ensemble de modifications profondes qu est la mue chez ces acariens on peut conclure de ces breves expériences que *Rhipicephalus sanguineus ne doit jouer aucun role dans la* [illegible] *et le maintien naturel de la peste*

Though in view of the evidence set forth above there can be no doubt that houseflies may become carriers of *P pestis* it is not at all likely that they then become apt to convey plague

Wayson [1] working with the stable fly *Stomoxys calcitrans* found that

"when applied to an animal (guinea pig) acutely ill of this disease or of plague bacteremia eight bites by one or more flies (not over two have been used simultaneously) will effectively transmit the disease to a healthy animal if the application is made within an hour after the flies have bitten the affected pig

In view of this experimental evidence one cannot exclude the possibility of a mechanical transmission of plague through the stable fly Certainly however actual instances of such a conveyance of the infection if occurring at all are exceptional

Gosio [86] reported that when fed on the organs of animals dead from plague the larvae of necrophilous flies harboured numerous plague bacilli and that these organisms were passed on to the pupae and adults in diminishing numbers Similar claims were also made by Russo [3] However interesting as these findings are they are of no practical importance as far as the transmission of plague is concerned

Mosquitos

The evidence incriminating the mosquitos and allied species of blood sucking insects as potential vectors of plague may be summarized as follows

(1) Bonnardiere & Xanthopoulides (quoted by Dieudonne & Otto [46]) found plague bacilli in the proboscis and stomach of an insect (Stech muecke) which had just bitten a plague patient

(2) Flu [63 64] was able to prove that specimens of *Mansonia Culex pipiens Anopheles rossi* and *Aedes aegypti* which had been fed on plague affected laboratory animals could harbour virulent plague bacilli for some days but did not transmit the infection through their bites

(3) Blanc & Baltazard [0] found that *Aedes aegypti* which had fed on plague infected guinea pigs harboured *P pestis* for ten days after the last infective meal but were incapable of transmitting the infection

Many observations made in China during the second World War proved that mosquitos even though they may become infected are unable to transmit plague Although the patients in the emergency hospitals were often covered with mosquito bites not a single case of plague occurred which could be ascribed to such bites With the exception of one medical officer who was infected during the day through a flea bite no instances of bubonic plague were noted among the staff of the hospitals These findings are in accord with results obtained by Hunter [113] who failed to detect plague bacilli in a group of 20 30 mosquitos which had been caught inside the nets covering plague patients in the Hong Kong Infectious Diseases Hospital

Triatoma

Mertens[171] explored the possibility that *Triatoma rubrofasciata*, an insect normally feeding on the rats of Java but occasionally attacking man, might convey plague. He found that guinea pigs inoculated subcutaneously with specimens of this insect which had been fed on plague affected animals became fatally infected, and that the insects if kept starving after an infective meal, could harbour *P. pestis* for at least one month. However, the faeces of infected *Triatoma* were free from plague bacilli. Carrying out transmission experiments with this insect, Mertens obtained only one positive result when using a *Triatoma* which bit a healthy guinea pig immediately after its meal on an infected animal had been interrupted. *Triatoma rubrofasciata* is therefore certainly not a dangerous plague vector.

Ants

Hankin[90] was able to demonstrate *P. pestis* in ants which had fed on the carcasses of plague rats, and to infect laboratory animals (rats and mice) with triturates of these insects.

In contrast to these findings, Russo[223] maintained that the virulence of plague bacilli harboured by ants was apt to become lost.

Beetles

Early experiments by Cao[33] showed that beetles which had been fed on plague material were apt to excrete virulent *P. pestis*.

Skorodumoff[237] working in Transbaikalia, was able to produce plague in a laboratory animal inoculated with a triturate of *Necrophorus dauricus* found on a naturally infected tarabagan. However, experiments performed with other specimens of this beetle species and with *Silpha perforata* collected from infected tarabagans gave negative results.

Russo[223] found that two species of necrophilous beetles—namely *Sitophilus* and *Tenebrio molitor*—could harbour *P. pestis* when fed on plague material.

Notwithstanding these positive findings it is altogether unlikely that beetles can play a role in the transmission of plague.

Cockroaches

Early observations on the presence of *P. pestis* in cockroaches were made by (*a*) Cao[33] and Kuester (quoted by Dieudonné & Otto[46]) who found that these insects, when fed on plague material could void virulent plague bacilli in their faeces, (*b*) Pound[206] who ascribed an instance of laboratory infection in a healthy guinea pig to young cockroaches which had had previous contact with plague infected animals and was able to prove this contention by bringing a second guinea pig into contact with such cockroaches, and (*c*) Hunter[113] who demonstrated the presence of *P. pestis* in cockroaches (*Blatta orientalis*) collected in plague houses.

However further observations indicated that the ability of cockroaches to harbour *P pestis* was not great Barber[10] who injected these organisms into the body cavities of 61 cockroaches (*Periplaneta americana* and *Rhyparobia maderae*) obtained only one positive result when making animal experiments with these insects Jettmar[1 8] found that (*a*) as proved by guinea pig experiments plague bacilli perished or soon lost their virulence in the alimentary tracts of cockroaches (*Blatta germanica*) (*b*) it was not possible to infect guinea pigs with fresh excrement from cockroaches fed on plague material and (*c*) inoculation of *P pestis* into the coxa stumps of an amputated leg did not produce plague in cockroaches

It is unlikely therefore that cockroaches play any role in the trans mission of plague Certainly however it is essential to keep premises where experimental animals are kept free from these pests because as shown by the observations of Pound and others young cockroaches have an almost uncanny ability to insinuate themselves into containers which seem impregnable to the inroads of insects

Conclusions

Dealing with the role of insects other than fleas in the transmission of plague Wu[91] expressed the opinion that rodent lice and ticks alone have to be taken into serious consideration but that compared how ever to the rodent fleas especially X *cheopis* the role of even these insects is of little import Indeed broadly speaking the terms insect vectors of plague and rodent fleas may be taken as interchangeable

Though under special conditions certain insect species other than the rodent fleas may play a comparatively important adjuvant role in general one ought to be in agreement with the dictum of Wu

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Chapter 8

CLINICAL ASPECTS

INCUBATION

The problem of the length of the incubation period of human plague received early and thorough attention on the part of the Indian Plague Commission As summarized by Simpson [175] the cases the Commission considered in this respect may be divided into three classes

Class I comprising instances in which there was a history of a direct inoculation of infective material as well as such where there was a history of the patient having come into contact with infection on a particular occasion In both groups there were cases where the incubation period could not have been longer than 24 hours and others in which it extended to five days The average length of the incubation period was about three days

Class II consisting of cases in which there was a history of the patient having been in contact with infection on and after a particular day The data gathered for this class confirmed those of Class I in placing the period of incubation between one and five days

Class III representing cases in which plague developed after removal from infected surroundings Out of 753 cases noted 15 (1 9) developed plague after the 10th day but it was stated that these patients might have contracted the infection after removal to the camp

In Simpson s own opinion

" The incubation period of whatever type the disease may be varies generally between a few hours and five days it being rarely longer Cases have been recorded with longer periods but it is often difficult to dissociate from them the possible exposure to infected clothes or infected animals at a date later than that which is believed to be the time of infection Still the evidence at present existing does not exclude the possibility of the period of incubation being prolonged occasionally to 12 or 14 days "

The plague subcommittee of the Office International d Hygiene Publique meeting at Paris in 1926 [147] maintained similarly that the duration of incubation of human plague did not ordinarily exceed 6 days in the case of direct infection by the respiratory route the duration of incubation did not generally exceed 3 or 4 days but it might be as long as 6 days in exceptional cases

Statements made in some recently published manuals on plague or tropical diseases regarding the length of the incubation period of plague may thus be summarized

Author	*Date*	*Length of incubation period*
Chun [20]	1936	Usually 3 5 days but may vary from less than 24 hours to 10 days or, in exceptional cases even up to 15 days
Joyeux [93]	1944	Bubonic plague 1 5 days but may be prolong d to 9 10 days Pneumonic plague on an average 2 6 days but may last only a few hours or may be prolonged to 9 10 days
McCoy [119]	1944	3 12 days in most cases 4 5 days
Rogers & Megaw [166]	1944	Usually about 3 days, but said to vary from 2 to 10 days rarely it may be extended as long as 15 days
Strong [188]	1944	Varies generally from 2 10 days but is usually 3 4 days Primary pneumonic plague may not exceed 2 3 days
Mackie et al [120]	1945	Bubonic plague 2 4 days less often 10 days Pneumonic plague may not exceed 2 3 days
Pozzo [158]	1945	Bubonic plague varies from 2 to 10 days but is ordinarily 3 4 days Primary pneumonic plague from 15 to 20 hours up to a maximum of 2 3 days
Napier [143]	1946	2 8 days rarely longer average about 4 days
Dubois & van den Berghe [9]	1948	2 9 days most often 3 4 days
Mathis & Pons [127]	1948	Bubonic plague 10 hours to 3 days Pneumonic plague 48 hours on an average but can vary from 1 to 5 days
Giordano [56]	1950	2 8 days
Manson Bahr [1 1]	1950	2 8 days rarely up to 15 days
Macchiavello [118]	1951	Usually 2 5 days with a maximum of 10-12 days Incubation periods of only 12 15 hours have been recorded in India

As will be gathered from the above summary, some disagreement exists regarding the minimal and maximal lengths of the incubation period of plague However, the various writers are agreed that the length of incubation of bubonic plague is as a rule less than 6 days which is the period contemplated in the International Sanitary Conventions of 1926/1944 and in the International Sanitary Regulations [1] adopted by the World Health Organization in May 1951

Most authorities state that the average length of the incubation period is even shorter in the case of primary pneumonic plague, but it should be noted that Chun [20] analysing the experiences made during the 1920 1

Manchurian epidemic at Harbin found an incubation period of up to 6 days frequent enough to deserve serious attention from an administrative viewpoint

Though one ought to share Simpson s opinion [1 5] that observations suggesting abnormally long incubation periods are apt to be fallacious no doubt can exist that instances of this kind do occur Reliable observations have in fact been recorded more recently by authors such as Durand & Conseil [3] Fonquernie [50] Calbairac & Seyberlich [19] and Girard [59, 61] who noted—in the case of primary pneumonic plague in particular—instances of abnormally long incubation lasting up to 10 days Consequently the period during which contacts with pneumonic plague patients were kept in the isolation camps of Madagascar was prolonged to 10 days There can be no doubt however that nowadays such a prolonged segregation of pneumonic plague contacts or possibly even their segregation at all has become unnecessary in view of the availability of methods of abortive treatment Application of the now available insecticides certainly renders the segregation of contacts with bubonic patients superfluous

SYMPTOMATOLOGY

General

The following clinical features because commonly met with in all severely affected plague patients regardless of the primary localization of the infection may be dealt with comprehensively the more so as they are due to an action of circulating plague toxins rather than to that of the causative organisms themselves

Onset and prodromal symptoms

There is general agreement that in the overwhelming majority of severe plague attacks the onset of the disease is sudden so that as aptly stated by Mathis & Pons [127] one is confronted in 95% of the cases by the clinical picture of a severe infectious condition of the type of a major septicaemia Prodromal symptoms if occurring may consist of malaise headache giddiness mental apathy or restlessness nausea and pains in the limbs or in the lumbar region Some of these symptoms particularly headache and nervous disturbances may persist or if they were not pre existent may appear in the course of the illness

While according to some observers the rapid rise of the temperature in the cases with a sudden onset is initiated merely by some feeling of chilliness or slight shivering others noted the presence of marked rigors Macchiavello [118] in particular, stated that plague began commonly with

a single prolonged intense chill, or with less severe or repeated chills accompanied by trembling or shaking' Such marked rigors were hardly ever observed in the plague patients of China Rogers & Megaw,[166] who gained their experience in India, also speak merely of chilliness when describing the onset of plague

In any case, the temperature rises rapidly, often reaching 103°F or 104°F (39 5° 40°C) in a few hours or at most, within a day The pulse rate increases at the same time and, as confirmed by observations of the present writer, the pulse may not only soon become more rapid than corresponds to the temperature, but may also commence to be dicrotic or even intermittent, quite early in the disease

Generally speaking, the condition of the patient deteriorates incessantly so that the disease may become fully developed within a day if not within a few hours

Course

Many authors particularly the early writers, maintained that plague patients showed a characteristic facial expression and considered this facies pestica as pathognomonic These statements cannot be considered to be generally valid, however, because as pointed out by some observers such as Muller & Poch,[141] the facial expression and the aspect of the eyes (Blick) of the plague patients are apt to vary markedly in relation to the state of their sensorium and the pains which they feel One must also agree with Muller & Poch that the facial expression noted in some of the sufferers is not characteristic for plague alone but may be seen in any patient suffering from a disease with ' typhous impairment of the sensorium '

However, necessary though it is to bear these restrictions in mind the general appearance of severely affected plague patients is often so characteristic that, at least during an outbreak, the presence of the disease may be suspected at a glance The sufferers appear to be struck down by a most serious illness and, even if they are restless instead of being prostrated, it can be seen that they are in a greatly weakened condition Except in the case of collapse the faces of the sufferers are as a rule flushed and sometimes bloated as well Early in the disease their countenance often depicts anxiety and distress but later their facial expression becomes resigned listless, and apathetic In patients whose sensorium is not free the eyes may be staring or may show aimless movements The conjunctivae are almost invariably injected often markedly so the nostrils are dilated and the lips are dry As long as fever is present the skin of the patients is hot and dry As noted earlier the pulse is rapid often more or less dicrotic becoming in the later stages of the disease feeble intermittent and finally imperceptible

A systematic examination of the patients reveals the following

Temperature Various types of fever—high continued remittent or irregular—may be observed in plague patients according to the form of the disease and the severity of the affection As aptly described by Simpson[175] the fever

> rises in the bubonic form to 103 104° F or may be to 105 F or to 106 F [39 5 40° 40 5 41 C] and may reach its highest on the evening of the first day and continue at its maximum but more usually it gradually rises reaching its maximum on the evening of the second or third and sometimes but seldom on the fourth day an intermission of a degree or more frequently taking place during a part of the day [usually in the morning] On the third fourth or fifth day the temperature usually falls 2 or 3 degrees or more continues at this low temperature for a few hours or a day and then rises again reaching nearly the same or a greater height than that of the previous evening If this stage is successfully passed through the temperature again falls the next day and then by successive evening exacerbations and morning remissions steadily comes down by degrees to normal or sub normal which may be reached on any morning between the sixth and eleventh day In simple bubonic cases of a mild character the temperature may fall to normal as early as the second or third day on the other hand the occurrence of complications or the eruption of buboes may cause great irregularity in the temperature and completely obliterate the more or less typical primary and secondary rise with the apyrexial interval

Successful treatment with modern remedies markedly shortens the febrile period leading to a gradual or sometimes even to a rapid fall of the temperature However even if adequate treatment is continued so as to avoid relapses suppuration of the buboes may lead to the appear ance of intermittent fever until the pus is evacuated

As described by Simpson[175] in septicaemic plague the temperature rises rapidly and running an irregular course generally remains high However in particularly severe cases the temperature may not rise above 100°F [37 8°C] or less in the early stage and it is only if the patient lives long enough for reaction to set in that there is any considerable rise in temperature

In the primary pneumonic form of plague the temperature is high but often runs an irregular course It may suddenly fall before the death of the patient

Circulatory system As noted above an incessant quickening of the pulse accompanied by a fall in blood pressure and irregularity of the pulse waves indicates the presence of progressive heart failure The pulse may finally become so rapid and imperceptible that it can no longer be counted Subjectively the patients may suffer from a feeling of oppression in the precordial region objectively dilatation of the heart and feebleness of the heart sounds may be noted as the disease progresses Sudden death from heart failure may take place not only during the acute stage of the

disease but also during convalescence following even slight exertions on the part of the patients

Most observers are agreed that as a rule severely affected plague patients show a marked polymorphonuclear leukocytosis, according to Bonebakker,[6] with a shift to the left The number of white blood corpuscles, though usually not exceeding 20 000 25 000 may reach 50,000 or even more Wagle & Colah [206] maintained in this connexion that an enumeration of the leukocytes is of prognostic importance, the prognosis being best in patients showing leukocytic counts within normal limits It is noteworthy however, that a leukocytosis may also be absent in pneumonic plague patients and in particularly severe cases of ' septicaemic plague Leukopenia may be present in this form of the disease and according to Wagle & Bedarkar [205] also in some patients with primary pneumonic plague

Contrary to the findings of other workers, Rogers & Megaw [166] claimed that in half of the plague cases seen by them differential counts showed a high proportion of lymphocytes during the first three days of illness and ascribed some differential diagnostic importance to this feature

It was also stated that in the early stages of plague the number of red blood cells and the percentage of haemoglobin are not rarely increased above normal (Rogers and Castellani, quoted by Strong [188]) After the attacks particularly if complications are present a more or less marked secondary anaemia may develop

In severe plague cases the presence of submucous haemorrhages may lead to bleeding from the stomach, the intestines or the bladder Bleeding from the nose is not uncommon

According to what has been stated in chapter 4, an initial bacteraemia is frequent even in apparently uncomplicated cases of bubonic plague but is of a passing character if the disease tends to run a benign course As a rule bacteraemia becomes established in seriously affected patients suffering from any type of plague It is usually progressive in character the number of bacilli in the peripheral blood becoming maximal in the agonal period Observations made in this connexion by the Plague Research Commission (quoted by Petrie [150] and by Girard [63]) showed that out of 74 blood samples taken from 28 plague patients five of whom recovered 30 were positive, but that in only six instances was it possible to find the bacilli in smears In seven of the 23 fatal cases less than 10 organisms per ml of blood were found Only five of the 16 other patients showed more than 1,000 organisms per ml of blood, and only two had more than 10 000 *P pestis* per ml

It is important to note that in the experience of subsequent workers such as Girard [63] and Pollitzer (unpublished observations) the number of plague bacilli in the blood of moribund patients was frequently found to be considerably higher than in most of the sufferers examined by the Plague Research Commission even blood smears often and quite markedly proving

positive Still general agreement exists that apart from rare exceptions the degree of bacteraemia in human victims is far below that commonly observed in rats succumbing to acute plague

The presence of a conspicuous bacteraemia usually renders the prognosis most unfavourable but it is noteworthy that recovery was observed by the Plague Research Commission even in a patient from whose blood 500 600 colonies of *P pestis* per ml of blood had been obtained

On the other hand as pointed out with much reason by Girard [63] in man as well as in experimental animals an overwhelming infection leading to marked toxaemia may result in rapid death before bacteraemia becomes conspicuous

Devignat recently postulated that bacteraemia was more marked in sufferers infected with *P pestis* of the variety *antiqua* (i e, strains acidifying glycerol and reducing nitrates to nitrites) whereas the character of the disease was less serious in epidemics caused by the glycerol negative "oriental variety of the plague bacillus Though no doubt the manifestations of human plague observed by Devignat in the Belgian Congo which were caused by *P pestis* var *antiqua* were of a most serious character the same held true for many outbreaks caused by the oriental variety of the plague bacillus

Respiratory system Though involvement of the respiratory tract is not peculiar to the primary pneumonic type but is almost invariably present in severely affected patients suffering from any form of plague the physical signs found on examination of the lungs are often rather inconspicuous This holds true not only in cases where merely some bronchitis or congestion of the lungs is present but often even for patients where a secondary or primary pneumonic process develops One might almost say that the marked discrepancy between the slight clinical signs usually found in the respiratory tract of the patients and the often marked lung involvement seen at autopsy is a characteristic feature of the disease

While the clinical aspects of plague pneumonia will receive further attention later it has to be noted here that terminal lung oedema leading to marked dyspnoea and cyanosis is practically always present in patients dying from any form of plague unless they suddenly succumb to heart failure To judge from the personal experience of the present writer lung oedema is particularly conspicuous in patients with an apparently primary invasion of the blood stream

Nervous system As maintained by Simpson [175] next to the local manifestations of the infection the most characteristic symptoms of plague are those connected with the nervous system Dealing in a masterly manner with the nervous phenomena observed in this disease he stated that

"The plague virus evidently produces a progressively intoxicating effect on the nervous system which displays itself with varying degrees of intensity in different ways on different

constitutions In some there is insomnia in others wild delirium in others stupor in all more or less loss of coordinating power over the voluntary muscles and dulling of the senses The staggering gait and the inability to coordinate the movements of the hands are very characteristic symptoms There is no paralysis of the limbs but from the physical weakness, vertigo and toxic impression on the nervous system the voluntary muscles are not completely under the command of the patient The speech is also peculiarly hesitating stuttering thick lisping indistinct and monosyllabic often like that of a drunken man The memory is confused and in answering questions the patient forgets half the sentence or syllable of the word which he began to utter

As will be gathered from this description, the nervous reaction of the patients to the plague toxin varies markedly In fact, some of the sufferers, particularly those with primary pneumonic plague remain conscious, *rational and with speech unaffected to the last Sudden transitions may* occur, patients who seem apathetic quite unexpectedly leaving their bed and trying to get into the open Such restlessness is, on the whole, most often seen in the later stages of the disease, apparently because the patients then suffer from air hunger"

Possibly regional differences exist in the nervous manifestations displayed by plague patients Macchiavello[118] maintained in this connexion that while delirium and stupor are the rule in India and China, in Africa and especially in South America, these symptoms are lacking in the great majority of cases No doubt however, individual differences and different habits of life are also of importance in this respect For instance, the few soldiers admitted to the plague hospitals of China appeared, as a rule, to be far more restless—or even boisterous—than the patients from the class of farmers or labourers who were usually stuporous or quietly delirious Nervous excitement was also comparatively less conspicuous in the female patients than in the males even though the latter were as a rule not addicted to alcohol Convulsions, which are not often observed in adult plague patients seem to be more frequent in children affected by the disease

It is consoling that pari passu with the increased use of the now available remedies, serious nervous disturbances as well as other grave symptoms of plague infection are now far more rarely seen than in the past

Digestive system Plague patients while usually having little or even no appetite, almost invariably feel intensely thirsty They are either constipated or have diarrhoea according to Mathis & Pons[117] frequently an initial phase of constipation leading to slight meteorism is followed by a terminal phase with diarrhoea

Statements as to the frequency of vomiting in plague are not unanimous some writers merely noting that it may be present others stating that it is frequent early in or even throughout the acute stage of the disease Macchiavello,[118] who considered nausea followed by vomiting as an early symptom of plague maintained that usually it was of nervous origin instead of being due to gastro intestinal causes

It is unfortunate that even in plague areas where vomiting is not a prominent symptom like in China it was quite frequently induced when sulfonamides were administered thus seriously interfering with treatment by the oral route

The tongue of a severely affected plague patient is usually swollen and protruded with difficulty in a tremulous manner It is at first coated with a creamy white fur the tip and edges remaining red Later the coating becomes dry and glistening and finally a yellowish brownish or even black coat is formed, similar to that seen in typhus or typhoid Lips teeth and gums may become covered with sordes If the patients begin to improve the tongue soon tends to become moist and clean so that its examination is of some importance for assessing the condition of the sufferers

Abdomen As a rule physical examination of the abdomen shows nothing characteristic The liver and also the spleen are usually moderately enlarged and the former organ may be tender particularly if heart failure becomes marked Occasionally general tenderness of the abdomen associated with meteorism and vomiting was found to be present

If the deep iliac lymph nodes are the seat of primary buboes marked pains and tenderness may be present in the corresponding lower quadrant of the abdomen If the process is localized on the right side a syndrome quite similar to that of appendicitis may be produced and cases are on record where patients showing such a localization of the buboes have been subjected to operation (Macchiavello [118])

It is also noteworthy that according to some observations of Goldstein [7] pneumonic plague patients may complain of pains not in the chest but in the abdomen He referred in particular to one sufferer who had such rigidity and guarding in the left epigastrium that I suspected an acute abdomen and asked the surgeon to see him There was nothing in the lungs and pleurae and it was only after two days that some crepitations at the right base were audible and the patient brought up blood stained sputum which contained *P pestis*

Genito urinary system In accordance with the presence of high fever the urine of severely affected plague patients is scanty and highly coloured It often contains traces or moderate amounts of albumin and if so hyaline casts are found

Haemorrhages in the urinary tract as they frequently occur may also lead to the presence of erythrocytes in the urine but marked haematuria though recorded by some writers appears to be exceptional

Chun [20] who examined the urine of a series of patients suffering from primary pneumonic plague noted in most instances a diminution of the chlorides but it is noteworthy that according to Simpson [175] the urine of

plague patients in general is deficient in chlorides as well as in urea and uric acid Instances of anuria have been described but appear to be exceptional Retention of the urine in the bladder of unconscious or delirious patients is more common

It is important to note that the urine as well as the faeces of severely affected plague patients may contain *P pestis*

Unless specific treatment is started early, serious attacks of plague in any form usually lead to abortion or miscarriage in pregnant patients events which exert a most unfavourable influence on the outcome of the illness

Termination

Unless adequate specific treatment is administered, patients suffering from primary pneumonic plague almost invariably die within a few days, sometimes even within less than a day The average length of life in 1 128 such victims seen in 1921 at Harbin Manchuria, was 1 8 days [9] Similarly patients suffering from the so called primary septicaemic form succumb when not energetically treated, almost always within 1 3 days, but sometimes even within a few hours (pestis siderans of the early writers) On the other hand, patients affected by benign forms of bubonic plague without bacteraemia almost invariably recover even if no treatment is given The average fatality rate in untreated patients suffering from the usual type of bubonic plague leading to bacteraemia is high amounting to 60% 90% in India and China

Though it may take place sooner or later, death during the acute stage of bubonic plague usually occurs within a period of 3 5 days from the onset of illness so that patients who survive for longer than five days have considerably increased chances of recovery

In most instances heart failure is the immediate cause of death Collapse may set in fairly rapidly or the patients may even succumb suddenly when making exertions In other cases there is a more or less prolonged period of agony marked by lung oedema and frequently also by oedema of the brain which leads to low delirium and/or coma

Occasionally, death during the acute stage of the disease may be due to the development of complications, particularly meningitis Marked oedema developing round cervical buboes may, in rare cases, lead to death from asphyxia

Recovery from bubonic plague is usually gradual The period of convalescence may be long and, as will be described when dealing with the devolution of the buboes serious or even fatal complications may develop during this stage As has been noted before even convalescent patients may succumb suddenly to heart failure

Bubonic Plague

For various reasons it is impossible to discuss the clinical aspects of bubonic plague as comprehensively as the pathologist is entitled to do when dealing with this form of the disease Frequently, the morbid process usually produced through the bite of insect vectors particularly rodent fleas leads not only to the appearance of buboes but also to a generalization of the infection characterized by the serious general symptoms previously described and often also by complications which deserve separate attention

FIG 28 INGUINAL BUBO



However benign cases are met where the infective process remains localized in the primarily invaded lymph nodes and owing to the absence of septicaemia general symptoms if manifest at all, remain inconspicuous On the other hand, an invasion of the blood stream may take place so rapidly that reactions on the part of the regional lymph nodes fail to become manifest and the clinical picture is that of a most severe generalized infection, often designated as primary septicaemic plague Though as stated in chapter 4 this type of the disease has no independent standing from the viewpoint of the pathologist, it presents clinical particularly diagnostic features of its own which render its separate discussion desirable

An entry of the infection through the superficial mucous membranes as well as the appearance of primary skin manifestations at the portal of entry of the infection produce peculiar clinical forms of the disease which also deserve special consideration

For these reasons it seems appropriate for the purposes of the present disquisition, to deal with the different types and varieties of bubonic plague separately

Buboes

Localization General agreement exists that, as a rule, groin buboes are most frequent (about 55%-70%) femoral buboes situated distally from Poupart s ligament being somewhat more frequent than inguinal ones Axillary buboes (about 20%) and cervical, particularly submaxillary buboes (about 10%) follow in frequency Buboes in other locations, such as those in the parotid region, below the clavicula near the elbow (epitrochlear buboes) and behind the knee (popliteal buboes) are rare Buboes under the subpectoral muscles are somewhat more frequent Though the primary buboes usually form at one site only bilateral and multiple locations have been recorded in a not inconsiderable minority of cases The following statistics which are based on large samples bear out these statements

	Choksy	*Observers Jennings*
Number of cases examined	9 500	16 132
Percentage incidence of buboes		
Femoral	30 87	30 79
Inguinal	23 25	24 23
Axillary	21 85	22 18
Cervical	8 40	9 11*
Other sites	1 68	—
Multiple	12 95	13 68

* Comprising besides 6 24 cervical buboes proper 2 87% of buboes situated in the parotid region

It is important to note, however that under certain circumstances axillary buboes or buboes on the neck may be more frequent than is

usually the case Axillary buboes are likely to occur in patients contracting infection in the foci of wild rodent plague where a transmission of the disease through direct contact with the affected animals is apt to take place Cervical buboes are apt to be frequent pari passu with a frequent occurrence of an entry of the infection through the faucial mucous membranes leading to tonsillar plague

Local peculiarities in the dress of the people probably also exert an influence on the distribution of plague buboes Thus Chun [20] pointed out that whereas in the experience of Choksy femoral buboes were more frequent than inguinal ones (30 87% as against 23 25%) among a series of patients observed in South Manchuria, inguinal buboes were preponderant (40% as against 8 7% femoral buboes) Chun ascribed this discrepancy in part to differences in the style of dress adopted by the Indians and the northern Chinese respectively In contrast to the Indians the Chinese not only wore shoes all the year round instead of using sandals or even going bare footed but also tied their trouser ends over close woven socks However since Chun s observations referred to 80 patients only no stringent con clusions should be drawn from his as well as from other experiences based upon small samples

Appearance and development As is unanimously stated in the literature and has been well borne out by the fairly ample field observations made by the present writer, manifestations on the part of the primarily affected lymph nodes appear as a rule quite early in the disease simultaneously with or even before the onset of the fever Some writers maintain that in a minority of cases the buboes appear later sometimes two three or even more days after onset of the disease However when one considers how inconspicuous the earliest manifestations in the affected lymph nodes often are one must wonder whether such an absence of early signs was more apparent than real

Even when seen within the first hours of illness as a rule the patients already complain of discomfort or even of some pain at the site of the future buboes Failing that it is almost invariably possible to prove that these sites are tender to touch The affected lymph nodes are at this stage but slightly enlarged and of normal consistency but from the first it can be definitely established that they in particular are the seat of the pain or tenderness to touch which the patients feel If as is often the case not one but two or several lymph nodes become primarily involved they enlarge at first separately so that at this early stage they can still be delimited by palpation

The further development of the local morbid process is subject to considerable variation In the most severe cases which are rapidly fatal the affected lymph nodes become but moderately or even slightly enlarged and show no marked periglandular involvement In the usual moderately

However, benign cases are met where the infective process remains localized in the primarily invaded lymph nodes and, owing to the absence of septicaemia, general symptoms if manifest at all, remain inconspicuous On the other hand, an invasion of the blood stream may take place so rapidly that reactions on the part of the regional lymph nodes fail to become manifest and the clinical picture is that of a most severe generalized infection, often designated as primary septicaemic plague Though as stated in chapter 4, this type of the disease has no independent standing from the viewpoint of the pathologist, it presents clinical, particularly diagnostic features of its own which render its separate discussion desirable

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The further development of the local morbid process is subject to considerable variation In the most severe cases which are rapidly fatal the affected lymph nodes become but moderately or even slightly enlarged and show no marked periglandular involvement In the usual moderately

severe type of the disease, enlargement of the lymph nodes progresses incessantly and development of marked periadenitic changes followed by infiltration and oedema of the surrounding tissues, leads within the first 12 hours of illness or even earlier, to the appearance of well marked buboes which become fully formed within one to five days after onset of the disease The size of a fully developed bubo usually varies from that of a walnut to that of a hen's egg, but smaller or larger sizes may be observed The buboes usually have an oval or round shape, their surface may be uneven if they are formed by the fusion of two or more lymph nodes Being at first movable the buboes soon become adherent to the surrounding tissues the skin becoming involved in this process if superficially situated lymph nodes become the seat of infection In such cases the skin loses its normal texture, appears smooth and tense and may show some degree of reddening, owing to the presence of acute inflammation Some early writers such as Simpson[175] state that haemorrhages, carbuncles, or blisters may appear on the skin covering the buboes and that the skin may even become gangrenous, but manifestations of this kind were rare in China and, according to Macchiavello,[118] also in South America

Palpation of a fully developed bubo reveals the presence of a doughy or boggy consistency in the outer layers of the swollen mass and a hard consistency in its central part formed by the affected lymph nodes

The subjective sensations produced in the patients by the presence of plague buboes may vary considerably, some complaining of constant dull or even stabbing pains, others feeling little or no local discomfort as long as they do not move and keep in a position which lessens pressure on the affected parts of the body Patients with a groin bubo keep for this purpose the corresponding thigh flexed those with axillary buboes lie on their back and hold the affected arm away from the trunk and those with cervical buboes hold their head still and inclined to the affected side

Generally speaking pain is more marked in the case of smaller buboes than in that of larger ones Accordingly, it may be present early in the disease but may disappear as the buboes increase in size Be this as it may, during the acute stage of the disease the buboes are invariably sensitive to touch often to such a marked degree that even comatose patients react when the affected lymph nodes are touched This marked tenderness to touch, which, as a rule, appears in the earliest stage of illness and is noticeable in the case of deep seated buboes as well as in those lying near the surface of the body forms one of the most outstanding clinical features of bubonic plague

Primary buboes of the secondary order, produced by an invasion of the causative organisms through the lymph channels, may appear in the vicinity of the initially affected lymph nodes In particular the intra abdominally situated iliac lymph nodes not rarely become involved in this manner If bacteraemia has become established, lymph nodes situated

away from the primarily affected ones in other parts of the body may become invaded by way of the blood stream As has been noted in chapter 4 the appearance of such secondary buboes does not as a rule lead to marked reactions in the surrounding tissues

Devolution The acute process leading to the appearance of primary plague buboes may subside in various ways Hand in hand with a gradual improvement of the general condition of the patients a return to normalcy may take place locally Often however this process is not complete the affected lymph nodes remaining somewhat enlarged and indurated the skin over such lymph nodes may remain pigmented

Frequently, suppuration commences in the centre of the bubo early in the second week of illness and an abscess is formed which barring surgical intervention is apt to open spontaneously In favourable cases the fistula thus formed closes after pus has been voided or sloughing has taken place for about two weeks and a scar is formed which varies in size according to the size of the abscess and the amount of sloughing However the process of healing may be delayed and sinuses may form which become secondarily infected In such cases chronic ulcers may develop and the patients may die some weeks later from sepsis exhaustion or amyloid disease (Napier [143]) Sad to relate cases may be observed in which too early or inadequate surgical intervention or unskilful after treatment of the wounds greatly retarded the process of healing or even led to serious consequences

Early writers referred to instances where excessive sloughing taking place in the course of suppuration led to the formation of large cavernous ulcers laying bare muscles nerves and blood vessels As pointed out by Simpson [17] these sloughing excavations were particularly dangerous when forming in connexion with iliac buboes in the pelvis because in such cases haemorrhage from pelvic arteries was apt to result Instances of this kind are rarely seen nowadays However Robic & Minec [16] referred in 1938 to a case where involvement of the iliac lymph nodes followed by abscess formation caused the death of a plague patient through haemorrhage due to ulceration of a pelvic artery on the 17th day after the appearance of the initial inguinal buboes

The formation of unusually large scars due to excessive sloughing or retarded healing may lead to chronic regional oedema (Villafañe Lastra & Rodeiro [0]) or in the case of femoral buboes to chronic oedema of the corresponding lower extremity (Downie [27] Phillips [151])

Hand in hand with the process of devolution, the causative organisms often first undergoing involution or becoming the prey of phagocytes disappear as a rule fairly rapidly from the buboes or from the pus which has formed However as will be discussed below some instances have been

observed in which *P pestis* continued to be present for weeks or even months

Complications

As Simpson[175] pointed out with much reason, it is an open question whether the secondary lung manifestations which frequently develop in the course of bubonic plague followed by septicaemia should be classed as complications or should be considered merely as extensions of the infective process so that 'from this aspect they form but a part of the disease' It is in accord with this concept that as stated in chapter 4 some involvement of the respiratory tract is usually, if not invariably present in bubonic infections followed by bacteraemia varying in degree from simple catarrh to grave forms of congestion and specific broncho pneumonia

It is often difficult to recognize the presence of secondary plague pneumonia Its appearance is apt to lead to a deterioration of the general condition of the patients, but such aggravations may take place for other reasons as well As noted before, even if bronchopneumonic foci develop the physical signs found when examining the chest of the sufferers are often inconspicuous The sputum of such patients may show an admixture of blood or may even assume an aspect similar to that met with in primary pneumonic plague, but in other cases the appearance of the sputum is uncharacteristic Laboratory examination of the sputum is also not an infallible means of establishing the presence of a secondary plague pneumonia because plague bacilli may be found in the expectorations of bubonic plague patients whose lungs are not seriously or even not at all involved Nevertheless, abundance of the causative organisms in the sputum suggests the presence of a secondary pneumonia

Some observers have recorded that a kind of 'marasmus' may develop after attacks of bubonic plague and this condition usually leads to death Such sufferers become emaciated feeble in body and mind, and unable to take food and getting into a typhoid condition gradually sink It would appear that the development of this condition is usually secondary to the complications arising in the course of devolution of the buboes which have been referred to above

The secondary manifestations observed on the skin and mucous membranes of bubonic-plague patients as well as the meningeal complications are described below, together with the primary manifestations of the infection in these organs

Skin manifestations

Primary cutaneous lesions representing reactions taking place at the portal of entry of the infection may appear in two different forms

(1) The morbid process developing at the site of infection may remain restricted to the formation of a usually small vesicle which is filled with a turbid serous fluid containing plague bacilli, and slight local lymphangitis. The vesicles soon break and no further reaction takes place on the part of the underlying or surrounding tissues.

Lesions of this kind are probably more often present than they are noticed, particularly so as they seem to be more frequent in benign than in grave cases of bubonic plague. In fact some authors described an "ambulant" type of plague in which the morbid process consists solely of the formation of such vesicles at the site of invasion and some local lymphangitis.

(2) The second type of primary skin plague consists of the development of carbuncles at the site of infection. As described by Simpson [1,3] this process commences by the formation of ecchymotic or petechial spots which

> "... rapidly increase in size and then rise in the form of blisters with or without umbilication, while the circumference becomes hard, swollen and inflamed. The blisters contain at first a clear serous fluid, which is later dark, sero-sanguinolent or haemorrhagic, and in the contents are plague bacilli. The blisters soon break and show at their base a moist, bluish red, inflamed and angry looking circular or irregular patch, which at this stage may dry up and go no further, or the inflammation may extend to the subcutaneous tissue causing a circumscribed or diffuse swelling, the centre of which begins in a few hours to necrose, *forming a leathery looking scab. From this centre the necrosis spreads* rapidly to the periphery. The result is the formation of indolent ulcers ... with hard and red overhanging margins."

To this excellent description it should be added that a ring of vesicles which contain pus and plague bacilli, and which tend to coalesce, often forms on the wall surrounding the plague carbuncles.

Primary plague carbuncles are usually small or moderately sized, their circumference rarely exceeding one or two inches (2.5-5.0 cm). They may form on different parts of the body according to the site of the infection; comparatively often they are situated on the wrists or ankles of the patients. The appearance of the carbuncles may coincide with or precede the formation of the plague buboes. General agreement exists that usually the development of such marked reactions at the site of invasion presages a favourable outcome of the disease.

Generally speaking, primary carbuncles develop only in a small minority of bubonic plague patients. However, as stated by Macchiavello [118] they were frequent in some Peruvian outbreaks though remaining totally absent in others.

Besides the above described primary lesions, secondary skin manifestations may develop in plague patients suffering from a severe type of the infection. As confirmed by the experiences of França [51] referred to in chapter 4, skin haemorrhages are frequently found in such cases if properly

FIG 29 INGUINAL BUBO



looked for Though sometimes restricted to the vicinity of the buboes they are apt to be present on other parts of the body as well The appearance of numerous petechiae or ecchymoses particularly early in the disease is an ominous sign

Carbuncles or necrotic processes developing without preliminary formation of blisters may become manifest in the course of bubonic plague usually as the result of a metastatic infection through the blood stream However, as noted by some observers carbuncle formation near buboes may be due to a retrograde infection through the lymph channels While

usually rare in recent plague epidemics such marked skin manifestations seem to have been of common occurrence during historical plague outbreaks particularly the Black Death and the great plague of London in 1665

Secondary carbuncles or necrotic ulcers of haematogenous origin may develop on all parts of the body They may reach considerable size the necrotic process sometimes laying bare muscles nerves and blood vessels involvement of the last mentioned may lead to serious haemorrhages Generally speaking however, the presence of solitary secondary carbuncles is a prognostically favourable sign

The appearance of generalized pustular eruptions (plague pox or plague variola) has been recorded by several observers According to Macchiavello[118] instances of this kind were frequent in South America particularly in Ecuador Martinez Vinueza[1 5] observing a series of 227 plague patients noted the presence of such generalized variolous manifestations in 26 patients 24 of these sufferers succumbed to the infection

Meningeal involvement

As summarized by Meyer et al[134] instances of plague meningitis or rarely, encephalomeningitis were observed by some of the early workers such as the Austrian Plague Commission[1] (one instance of meningitis out of a total of 80 plague cases) the German Plague Commission[51] (3 cases of meningitis and one of encephalomeningitis out of a total of 376 cases) Calmette & Salimbeni[13] (2 cases of meningitis and 1 of encephalomeningitis a child showing a groin bubo and signs of meningeal involvement recovering under serum treatment) Durck[30] (primary pneumonic plague complicated by purulent leptomeningitis) No details are available regarding the cases reported by Godinho[71] and Sanhueza[169]

More recent observations recorded by Meyer et al[134] Landsborough & Tunnell[101] and others may thus be summarized

Author	*Date*	*Number of observations*	*Summary of findings*
Crowell[22]	1915	2 out of 75 cases	Died after illnesses of one month and two weeks respectively At autopsy purulent meningitis complicating bubonic plague
Lafont et al[106]	1915	1	Meningeal process considered to be primary in nature Died 24 hours after admission
Sheldon[173]	1915	1	Meningeal process considered to be primary in nature Died after 36 hours Diagnosed only by smear examination
Levy[109]	1920	1	Meningitis following bubonic manifestations in serum treated patient

Author	Date	Number of observations	Summary of findings
Nogue [146]	1923	1	Signs of meningeal involvement developing about three weeks after patient had been admitted with diagnosis of pneumonia Suspicious bacilli found in lumbar punctate and also in punctate of an inguinal lymph node Died a few days later showing at autopsy *P pestis* in smears from the lungs bronchial lymph nodes and liver Cranium not opened
Uriarte [106]	1924	1	Bubonic plague complicated by unilateral iridocyclitis and hypopyon Progressed favourably under serum treatment but suddenly died three weeks later, showing at autopsy abscesses containing *P pestis* in the congested meninges
Paso [148]	1925	1	Serum treated bubonic plague patient who later developed signs of meningitis and died on 15th day of illness
Williams [209]	1934	1	Meningeal process said to be primary in nature
Montagne & Rivoalen [139]	1936	1	Patient with axillary bubo treated with serum on the fourth fifth and sixth days of illness Meningeal symptoms appeared on the 10th day of illness the patient dying four days afterwards
Meyer et al [134]	1937	1	Chronic relapsing plague meningitis in boy originally suffering from bubonic plague Died four months after onset of illness No specific treatment
Burton & Hennessey [30]	1940	1	Child with skin abscess which healed after incision and two days sulfapyridine treatment Meningeal symptoms appeared after two attacks of fever treated with sulfapyridine on the 25th day of illness Death occurred four days later Two guinea pigs inoculated with cerebrospinal fluid died after 11 and 14 days respectively
Lewillon et al [110]	1940	1	Admitted with signs of apparently primary plague meningitis on fourth day of illness died four days later Plague bacilli found only in cerebrospinal fluid not in material obtained through puncturing heart liver and lungs
Villafane Lastra & Rodeiro [20]	1942	4 out of 39 cases	Patients with meningeal involvement complicating bubonic plague who succumbed though given large doses of sulfathiazole
Koenigsfeld & Nambiar [99]	1946	2	Patients with axillary buboes who improved after initial treatment with sulfonamides but showed signs of meningeal involvement three weeks and one month respectively after onset of illness The first mentioned patient died one day later the second two days later

Author	Date	No. of observations	Summary of findings
Landsborough & Tunnell[101]	1947	8 out of 203 cases	With exception of one case considered as an instance of primary plague meningitis meningeal involvement appeared in patients suffering from bubonic plague at the earliest on the 9th and at the latest on the 17th day of illness Though treated with sulfonamides and serum all eight patients died the length of illness varying from 12 to 29 days
Videla[200]	1947	1	A patient considered to suffer from primary plague meningitis recovered when given streptomycin intramuscularly as well as intracisternally
Fain et al[42]	1951	1	Child showing on admission fever headache and evidence of malaria infection Initial treatment with quinine sulfathiazole and penicillin led after eight days to the disappearance of fever However temperature rose again one day later and signs of meningeal involvement appeared 12 days after admission when blood culture proved positive for *P pestis* Rapid cure through streptomycin administration

Remarks

(i) No details could be elicited regarding the instances of meningeal involvement recorded by Veintemillas[199] Mealla[125] and Kamal et al[95]

(ii) The recovering patient observed by Singh[178] probably suffered from meningismus and not from meningeal plague

(iii) Three instances of cerebral plague in which symptoms like unconsciousness delirium or (once) epileptiform attacks ascribed to the presence of brain oedema were present were recorded by Wright[214]

The symptomatology of meningeal involvement caused by *P pestis* closely corresponds to that observed in cerebrospinal fever or other acute cases of meningitis Headache painful stiffness of the neck, and presence of Kernig s sign form early and prominent features of the process Less common symptoms observed by Landsborough & Tunnell[101] in their series of eight cases included convulsions and affections of the cranial nerves (twice respectively) and vestibulo cerebellar symptoms deep coma and vasomotor crisis (once respectively)

As will be gathered from the above tabulation it was claimed that in a number of instances the meningeal process produced by *P pestis* instead of developing secondarily in patients with bubonic (or exceptionally with pneumonic) plague was primary in nature It is conceivable that in contacts of patients suffering from primary pneumonic plague a primary meningeal process might be produced through droplet infection However no evidence has been brought forward to prove that in the patients referred to the meningeal process was due to this mode of infection and not to an invasion of the blood stream by the causative organisms

" the original establishment of cerebral foci undoubtedly occurring during an early bacteremia did not lead to immediate multiplication of organisms and death but rather to a persisting of bacilli in a protected location where slow multiplication could be successful It might be that an infection with a strain of uniformly high virulence could lead to the fulminant meningitis while strains with a heterogenous population containing many low virulent organisms might give rise to the picture seen in California

Burton & Hennessey [10] considering their observations as well as those of Uriarte [196] and of Montagne & Rivoalen [139] expressed the opinion that in these cases particularly in their own the previous specific treatment might have influenced the invasive power of the causative organisms so that the reaction between them and the host might have become altered

Koenigsfeld & Nambiar [99] maintained similarly that in both their cases,

" the meningeal signs occurred in the course of a prolonged illness after some initial improvement Both patients seemed to respond favourably to sulpha drugs and succumbed only at a comparatively late stage of the disease Sulphadiazine probably lessened the virulence of the germ to a certain degree without rendering it entirely harmless with the effect that the disease took a more chronic course and meningitis had time enough to develop If this conclusion is correct more cases of plague meningitis may be observed in the near future as sulpha drugs are now in general use in the treatment of plague "

No doubt can exist that insufficient or delayed administration of plague serum or sulfonamides is incapable of preventing the appearance of late meningeal manifestations Pending further investigations it is difficult to decide whether the mechanism at work is an abatement of the virulence of the invaders or—as seems more likely—their persistence and slow multiplication in a protected location

Eye involvement

As proved by several well authenticated observations the eye may serve under natural as well as under experimental conditions as the portal of entry of plague infection Mizuo [137] who in 1910 made a special study of eye plague entitled Über die Augenveranderung bei Pest maintained in this connexion that entry of *P pestis* into the eye could lead to a generalized infection with or without production of a local reaction Sharing the opinion reached on experimental grounds by the German Plague Commission [51] he also expressed the view that the bacilli entering the eye were carried through the nasolacrymal duct into the nose and afterwards gaining access through the nasal or faucial mucosa reached the lymph nodes However since according to Mizuo s observations plague bacilli were apt to multiply rapidly in the conjunctiva particularly along the lymph channels no doubt can exist that they can reach the regional lymph nodes directly from the eye

The following instances of primary eye plague could be found in the available literature

(1) One case recorded by several workers concerned a nurse working in the Parel Hospital Bombay during the 1897 outbreak who received in the eye a particle of sputum

coughed up by a pneumonic plague patient Though the eye was carefully washed conjunctivitis set in on the next day which was followed by a swelling of the parotid a bubo below the ear on the affected side and death (*Simpson*[175])

(2) According to Simpson[175] a similar case occurred in Hong Kong

(3) As quoted by Mizuo[13] Hasegawa[9] observed *in 1900 an instance of plague conjunctivitis with symptoms resembling those of conjunctivitis gonorrhoica the patient in question died on the day of admission*

(4) Mizuo[137] also referred to the case of a Japanese doctor who working during an outbreak in 1902 in the Tokyo Plague Hospital developed a primary dacryocystitis followed by generalized plague infection with marked cerebral symptoms and died According to Mizuo this was the only case of primary plague dacryocystitis on record

(5) Jorge (quoted by Wu Lien teh[216]) observed during the 1905 outbreak at Oporto Portugal a plague patient who first showed signs of conjunctivitis and then a cervical bubo but recovered In this instance also infection seemed to have been due to an entry of pneumonic plague sputum

(6) A 2 year old child admitted in 1906 to the Osaka Plague Hospital Japan, with a history of slight trauma (contusion) of the right eye showed signs of acute plague conjunctivitis and two days afterwards also swelling of a pre auricular lymph node and the parotid gland on the corresponding side The infection became generalized and led to death on the fifth day after admission (*Mizuo*[137])

(7) A 14 year old girl admitted during the 1906 Osaka outbreak *with signs of plague* conjunctivitis also died on the fifth day after admission—apparently without development of a bubo (Mizuo[137])

It would be rash to claim that the above list is complete For instance the present writer was verbally informed that a further case of primary conjunctival plague had been observed during one of the recent epidemics at Foochow, South China Nevertheless it seems safe to state that primary *eye plague is a rare form of the disease*

It is important to note that, on the contrary, secondary eye affections, due to an invasion by the blood stream are by no means infrequent Mizuo[137] who, as stated above, made a *special study of this subject* noted their presence in 4 3% of his patients adding that quite frequently both eyes became affected

As summarized by Simpson,[1] the eye complications met with in plague ' may range from a simple inflammatory state *to one which is accompanied by ulceration of the cornea*, by copious haemorrhages and in some cases total destruction of the eyesight In Mizuo s[137] opinion panophthalmitis and keratitis were comparatively the most frequent complications followed by ring abscess of the cornea *corneal ulcer iritis*, and conjunctivitis Pozzo[156] considered a co existing affection of the iris and the choroid (iridocoroiditis) more common than keratitis or iritis (iridocyclitis) and added that the presence of retinal haemorrhages and optic neuritis had been observed in plague patients

As had been noted earlier (see page 412) congestion of the conjunctival blood vessels is a typical sign in severely affected plague patients Mizuo[137] maintained that some degree of conjunctival congestion was almost

FIG 30 INGUINO-CRURAL BUBO



invariably present regardless of whether or not the face of the sufferers was flushed Yamagiwa [0] even upheld that congestion of the conjunctiva bulbi was one of the three characteristic signs of bubonic plague the other two cardinal signs being a rapid rise of the temperature and the appearance of painful swellings in peripheral lymph nodes [a]

Referring to the fact that owing to intense congestion the conjunctiva bulbi shows a uniformly red hue in some plague patients Muller & Poch [141]

[a] D s plötzl he Anste gen der T mperatu d e s hme haft Anschw llung d r pe ph risch g leg n n Lymphdrusen u d d e Hyper m e d r Conjunct b lb s nd d e d charakte tisch n Zeichen f r die B bon pest

coughed up by a pneumonic plague patient Though the eye was carefully washed, conjunctivitis set in on the next day which was followed by a swelling of the parotid a bubo below the ear on the affected side and death (Simpson [175])

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It would be rash to claim that the above list is complete For instance the present writer was verbally informed that a further case of primary conjunctival plague had been observed during one of the recent epidemics at Foochow, South China Nevertheless it seems safe to state that primary eye plague is a rare form of the disease

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As had been noted earlier (see page 412) congestion of the conjunctival blood vessels is a typical sign in severely affected plague patients Mizuo [137] maintained that some degree of conjunctival congestion was almost

FIG. 70. ...



invariably present regardless of whether or not the face of the sufferers was flushed Yamagiwa [0] even upheld that congestion of the conjunctiva bulbi was one of the three characteristic signs of bubonic plague the other two cardinal signs being a rapid rise of the temperature and the appearance of painful swellings in peripheral lymph nodes [a]

Referring to the fact that owing to intense congestion the conjunctiva bulbi shows a uniformly red hue in some plague patients Muller & Pöch [141]

[a] D plötl h An te gen d r Tempe tu d s hm rzhafte An chwellung d r pe pherisch g l gen n Lymphd use und d Hypera m e d Co jun ti bulb s d d e d i cl rakte i chen Ze hen f r d e B bon npest

plausibly postulated that this was the reason why early observers often spoke of the 'wild look' (wilder Blick) of the sufferers

Tonsillar involvement

As set forth in chapter 4 the tonsils may either become the portal of entry of plague infection or may become secondarily involved in the course of attacks of the disease

A primary invasion of the faucial organs by *P pestis* may lead to the formation of tonsillar buboes, but instances of this kind seem to be rather rare One case was recently mentioned by Kamal et al[9]

In a second group of cases, entry of the infection through the faucial mucosa is followed by a local reaction in the tonsils and almost invariably also by the appearance of cervical buboes The intensity of these morbid processes may vary considerably There may be only swelling and more or less intense congestion of the tonsils but in other cases the local signs may be similar to those of follicular angina or they may even be characterized by the formation of ulcers or of membranes similar to those seen in diphtheria Cases simulating the latter disease were recently recorded by Wright[214] and by Magrou[1 3] In Wright s case diphtheria bacilli as well as *P pestis* were isolated from the tonsils , the patient, who showed no manifest bubo developed secondary plague pneumonia and died

The secondary involvement of the cervical glands may sometimes lead to a phlegmonous process similar to that evolving in Ludwig s angina One of the four cases of 'tonsillar' plague described by Wright[14] fell in this category

Instances of recovery have been reported in the case of patients showing only signs of tonsillar inflammation (de Souza,[187] Wright[214]) , as a rule plague bacilli were rare in the sputum of such patients Serious tonsillar involvement usually leads to secondary pneumonia and a fatal issue

Generally speaking instances of the above described anginous or tonsillar form of plague are rare However, as reported by Martinez Vinueza[1 6] and recently by Martinez,[1°5] cases of this kind usually followed by secondary pneumonia and death are quite frequent among the Indians of Ecuador who are in the habit of killing fleas and lice by catching them with their teeth

Secondary involvement of the tonsils is as a rule the result of a spread of the infection from adjacent cervical buboes through the lymph channels Marked oedema, involving the glottis may develop in such cases and lead to suffocation of the patients

Gastro intestinal involvement

Discussing the supposed existence of a primary form of gastro intestinal plague, Dieudonne & Otto[6] aptly stated that

"a primary infection of the gastro-intestinal tract, which Wilm and Galeotti believed to have observed repeatedly in Hong Kong, has not been seen by any of the Commissions working in Bombay; in any case this form is so rare that great caution must be exerted in accepting its existence. The absence of true primary buboes in the mesenteric lymph nodes, which would be a sine qua non to justify belief in such a form of plague, speaks against the existence of a primary gastro-intestinal type. According to the experiences made in the case of experimental animals, a very massive and direct invasion of the stomach or intestines would be necessary to produce such an infection. However, as shown by the observations made in one case by Trautmann & Lorey [1905], occasionally enormous numbers of *P. pestis* are found in the intestine of plague patients." *b*

The disbelief of Dieudonné & Otto in the existence of a primary gastro-intestinal form of plague is fully endorsed by the absence of recent reliable observations referring to such a type of the disease. However, as noted before, gastro-intestinal complications, such as vomiting and diarrhoea, are frequently present in severely affected plague patients and may become conspicuous. The appearance of submucous ecchymoses may lead to haemorrhage into the stomach or intestines, so that a usually slight or moderate amount of blood in the vomits or stools of the patients is not uncommon. Meteorism may be present and, as noted before, may be associated with tenderness of the abdomen.

The presence of jaundice in plague patients has been mentioned by a few recent observers only, such as Williams,[m] Blanchard et al.,[4] Bonebakker[6] and Macchiavello.[11] The last mentioned author stated that

"In Brazil in 1941 we saw an amarillic form [of plague] apt to be confused with yellow fever because of the icterus, the color of the vomitus, and the general symptoms which were especially violent in the digestive apparatus (hemorrhages, hematemesis, melena, vomiting and violent diarrhea) with complete absence of buboes."

The unusual plague case with toxic jaundice of the yellow fever type seen by Williams[09] in East Africa was apparently similar in nature.

Benign forms

Two main types of benign plague caused by an entry of the infection through the skin appear to exist. The morbid process may remain restricted to the site of invasion and its immediate vicinity or regional buboes and often also a slight or at most moderate general reaction may develop.

The first mentioned type, called "ambulant" plague by Napier,[143] is characterized by the formation of a vesicle at the site of infection and slight local lymphangitis. Constitutional signs remain absent.

Cases of this "ambulant" form of plague appear to be infrequent, but it must be borne in mind that they might be easily overlooked.

b Ein p m M gen u d Darm nf ktion be m Me chen d e *Wilm* u *G l ottl* in Hongko g oft rs g h zu h be m nt n wu d vo k ner der n Bombay a wes nd n Komm on n beob htet s e i t jed falls so selt n d ss m m t de An hm n r s l h n s hr vorsi ht g s n mus D geg n sp ht schon da F hlen n s r ht g n primären B bo n den Me e ter ald se w s d e te Bedingung fur d Ann hme ei er primaren D mpe t wäre Fur e ne ol he Infekt on w rd s l den T erv rs chen j denfalls hr g osser Me g n von Iestbac llen bed fe w lche d ekt in den Mag n der Dar n gel ngen m se And rs ts lehrt d r von *T* u *Lo ey* beoba htete F ll da unt r Umständ m Darm K ank Pestb ll n n ormer Ma se v rk mm n k n n

The clinical picture present in the second type of benign bubonic plague, which is usually designated pestis minor, may vary considerably In fact it is not possible to draw a sharp line of distinction between cases of this kind and those of the usual type of bubonic plague in which bacteraemia does not become established or marked

In the mildest variety of pestis minor one or a few lymph nodes (usually those in the groin, but sometimes those on the neck or in the axilla) become somewhat enlarged and tender to touch Pain in the affected lymph nodes if present at all, is not marked A slight increase of the temperature and malaise may be present for a few days, but the condition is usually not serious enough to induce the patients to lie down Attacks of this kind are therefore often designated "ambulatory" plague

In more-marked cases of pestis minor the affected lymph nodes may be slightly or moderately painful, but signs of marked inflammation and particularly reactions in the surrounding tissues remain absent Slight or moderate fever, headache, and some prostration are present and there may also be some conjunctival congestion However, these acute symptoms do not persist for longer than about a week, and even during this period the patients are up and about for most of the time or even are not bedridden at all

The affected lymph nodes may apparently become completely healed or may remain indurated or may suppurate

No agreement exists as to the frequency of pestis minor While some observers considered this form of plague to be rare others maintained that cases were frequent, particularly at the onset and at the end of outbreaks It must be admitted that since pestis minor patients rarely seek medical relief, attacks of this form of plague may be easily overlooked On the other hand, one must fully agree with Rogers & Megaw [166] that in localities where plague is expected there might be a tendency to suspect pestis minor whenever instances of gland enlargement are found even though they might be due to other causes Rogers & Megaw postulated therefore, with great reason, that in all doubtful cases of pestis minor a laboratory confirmation of the diagnosis is indispensable

It would appear that pestis minor was more frequent in some plague outbreaks or plague areas than in others Macchiavello,[118] for instance maintained that "epidemics of ambulatory plague have been observed in the north east of Brazil and in Ecuador" In an earlier report on plague in north eastern Brazil,[116] he stated that benign plague was called there "ingua de frio" (cold bubo) and also "febre de caroço" (stone fever), though the latter term was originally applied to ordinary bubonic plague He added that "ingua de frio

> appears generally in children under 15 and is characterized by mild and transitory symptoms monoglandular swellings without much inflammation or pain and a tendency of the gland swelling to become ligneous and to reoccur or to be readsorbed It appears

sporad all where plag e is end m and tend to d sappear when ep d m c There is no special rela on h p between c ses though they occas onally appear in small foci In some of these foci the first cases are severe and even fatal the later ones becom ng increa ngly m ld r There is a h s ory of previo s plag e in rats s an I it is poss ble that th s type of plag e may ha e some relat on to an attenuated v r s in fl as"

It is of interest to add that Kamal [84] during a plague epidemic in Egypt also found a number of children who showing enlarged and sometimes tender lymph nodes but no other signs apparently suffered from ambulatory plague

FIG 31 CERVICAL BUBO



Dealing with the problem of pestis minor, Mathis & Pons[177] pointed out with great reason that "even though a plague patient may be up and about at the onset of the disease, one should not consider the prognosis as favourable In such cases the appearances are often fallacious and the patient may succumb in a few hours" [c]

A few observers, such as Ilvento & Mazzitelli[86] Leger & Baury,[106] and Nikanoroff,[244] reported the presence of plague bacilli in slightly enlarged but not inflamed lymph nodes of healthy individuals, animal experiments carried out in most of these instances proved the *P pestis* strains in question to be fully virulent Whether it is legitimate to consider these persons as healthy carriers of *P pestis* as the above mentioned observers did seems questionable True enough the individuals concerned had no history of a manifest attack, but it is difficult to believe that the invasion of their lymph nodes by virulent plague bacilli produced no reaction whatsoever The proposal of Leger[104] to label such instances as cases of pestis levissima deserves, therefore, serious consideration It would seem that Sice[174] actually observed a case of this mildest form of bubonic infection He concluded that the individual concerned "was not a healthy carrier because of the attenuated reaction in the involved lymph nodes" [d]

Chronic forms

Instances of chronic bubonic plague have been described by a few authors Simpson[170] referred in this connexion to cases

> in which the disease runs a chronic course from the commencement The patient may walk about notwithstanding a certain amount of indisposition and catarrh and yet succumb later to the disease and be found the subject of abscesses containing plague bacilli in the lungs liver and spleen This chronic type closely resembles that found in lower animals

According to a statement made by Rebagliati[163] in 1939 atypical cases of plague polyadenitis with a prolonged course and subfebrile temperatures were met with in Peru

A similar syndrome was discovered in 1941 by Macchiavello[116] in northeastern Brazil As he summarized in 1951,[118] this multiglandular plague fever was

> characterized by a septic fever curve attenuated plague bacteriemia and a secondary septicemia caused by nonvirulent microorganisms The course is prolonged for a month or more during which time there appear successively from 4 to 14 or more variously situated large buboes There are alopecia cachexia and slow recuperation with a prolonged convalescence or death may follow

Macchiavello[118] also referred to a chronic benign form of plague in northeastern Brazil in which

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c " le fait que le malade continue au début de son infection à marcher et à se livrer à ses occupations *n autorise pas à porter un pronost c favorable* Il ne s ag t le plus sou ent que d un aspect trompeur et le sujet peut etre enlevé en quelques heures "

d " ce n éta t pas un porteur sa n, étant donné la réaction ganglionna re atténuée qu il présentait

the buboes whether suppurated or not or whether softened or woody showed periodic inflammatory exacerbations which did not interfere with the patients occupations although they caused a certain degree of debility and anemia

A case of chronic plague recorded by Durand [33] in 1931 appears to have been similar in nature

It is of importance to add that a few observers such as Choksy [18] Dujardin Beaumetz & Joltrain Durand & Conseil [35] Leger & Lhuerre [10] and Vacedes [1] found convalescents harbouring *P pestis* for periods of up to 12 months (one case of Durand & Conseil) in the primarily affected or even in secondary invaded lymph nodes In some of these instances particularly that described by Leger & Lhuerre it is not easy to decide whether the individuals concerned had actually become convalescent carriers or continued to suffer from benign chronic plague The line of distinction between these two conditions seems not well defined

Fulminant forms

As has been stated earlier only a difference in degree and not one in kind seems to exist between (*a*) instances in which an entrance of the infection through the skin or the mucous membranes leads merely to a slight reaction in the regional lymph nodes followed by a rapid entrance of the causative organisms into the blood stream and (*b*) the so called primary septicaemic form of plague where clinically manifest reactions in the lymph nodes remain absent and the fulminantly progressing morbid process seems to produce from the first a most serious generalized infection

A recent reviewer while accepting the classification of plague in two main forms only namely (1) bubonic plague including the septicaemic type and (2) primary pneumonic plague pointed out with much reason that the former name was hardly suitable to designate cases of the disease in which buboes were incapable of detection He proposed therefore the terms zootic and demic to designate the two above mentioned forms of plague However desirable though it would be to adopt this terminology it would seem rash to make general use of these startlingly new names in this monograph

There seems no need to enter at the present juncture into an exhaustive description of the clinical features met with in the fulminant types of bubonic (or one should rather say zootic) plague because the symptoms and signs of a most severe generalized infection which dominate or even quite overshadow the clinical picture in such cases have been fully dealt with earlier As a rule high fever is present in the early stage of the disease but as has been noted particularly severe and rapidly fatal cases are met with in which the sufferers fail to react even in this respect The activity of the heart is impaired from the first and deteriorates incessantly as the disease progresses Lung oedema often becomes marked in the terminal stage Delirium and/or coma are commonly present

Skin haemorrhages often become conspicuous and owing to the presence of submucous haemorrhages the stools, which as a rule are diarrhoic, and also the urine may show an admixture of blood

As a rule the patients suffering from what is commonly called primary septicaemic plague succumb so rapidly that there is no time for the development of complications However, some authors, for instance Wright,[215] have stated that such patients can survive long enough to develop pneumonic foci, and it was sometimes claimed that they were then apt to produce primary pneumonic infection in their contacts Claims of this nature ought to be viewed with scepticism, both because some of the authors seem to have made no clear distinction between secondary and apparently primary plague septicaemia and because the presence of lung oedema rather than that of pneumonic foci might have been responsible for instances of respiratory infection in contacts of such patients However in view of the fact that no sharp line of distinction exists between cases with ill defined buboes and those where no manifest reaction can be detected in the lymph nodes one should not be categorical in denying the possibility that lung or other complications might develop in patients apparently suffering from primary septicaemic plague

Until, recently, antibiotics became available for the treatment of plague only few instances of recovery from the primary septicaemic form have been recorded Moreover, as aptly stated by Robic during the discussion of a case reported by Le Gall et al [103] one should not be rash in diagnosing this form of the disease because with the aid of the now available refined methods of blood cultivation the presence of a few plague bacilli in the blood has been detected In Robic s opinion "one is entitled to speak of septicaemia only when the defence mechanisms of the body can no longer prevent the multiplication of the organisms in the blood [e]

This consideration ought to be kept in mind when evaluating the statement of Macchiavello [118] that it has recently been possible

> to confirm the existence of benign or ambulatory cases of plague septicemia (Peru) characterized by the absence of buboes a feeling of intoxication oneiric delirium and at times fever which does not last more than one or two days The usual diagnosis of these patients is benign grippe but blood cultures revealed the presence of very attenuated strains of plague bacilli

Primary Pneumonic Plague

Clinically, as well as pathologically three main types of primary pneumonic (or, one should rather say pulmonary) plague may be distinguished

(1) A type characterized by the development of well marked pneumonic foci (typical primary pneumonic plague)

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[e] Il n y a septicém e que lorsque la défense de l organisme ne s oppose plus à la mult plicat on et à la pullulation des germes dans le sang

(2) A transitory form with slight pneumonic lesions

(3) A form in which acute congestion and usually lung oedema are marked but where though plague bacilli abound in the deeper respiratory tract and in the lungs no consolidation is present in the latter

The clinical features present in these three types may thus be described

Typical form

It is important to note that even though typical primary pneumonic plague is characterized by the evolution of more or less marked lung involvement the disease is ushered in by a period lasting 20-24 hours during which symptoms and signs of a serious generalized infection predominate or are often even the only symptoms present

The onset of this initial " closed " stage of the disease is usually sudden High fever appears quickly often initiated by a feeling of chilliness or slight shivering far more rarely by marked chills As in severe attacks of bubonic plague the pulse soon becomes rapid and often also dicrotic The tension of the pulse may become lowered even in this early stage of the disease and some arhythmy may become manifest (Durand & Conseil[3]) The patients feel prostrated and complain of headache which often becomes intense However signs of involvement of the respiratory system are still insignificant or even altogether absent there is but little or even no cough expectoration if present at all is scanty and uncharacteristic smear examination of the sputum or saliva shows few if any suspicious bacilli

About 20 to 24 hours after onset of the disease signs of lung involvement begin to become manifest Cough appears or gets more frequent and leads to the expectoration of a sputum which may at first be purely mucoid or muco purulent but soon shows an admixture of specks or streaks of bright red blood In typical cases considerable amounts of a uniformly pink or bright red coloured sputum which may show a uniform aspect and a consistency comparable to that of raspberry syrup or may be foamy are soon voided Though sometimes becoming jelly like or even rather thick and viscid the sputum of pneumonic plague patients never reaches the degree of viscosity which is customarily observed in croupous pneumonia (Kasai[98])

Tachypnoea and dyspnoea are as a rule present and become more marked as the disease progresses but though the patients may complain of pain and a restricted feeling in the chest they usually seem to suffer far less than one would fear Summarizing his experiences in the 1920 1 Manchurian epidemic Chun[9] stated in this connexion that

There is not much pain in connection with plague pneumonia in contrast to ordinary pneumonia though a few exceptions were observed Nearing death the patient is very short of breath mildly delirious often tries to sit up or struggles to go out into the open air He is not conscious of much suffering owing to the impaired cerebration "

The physical signs found when examining the chests of the suffere are often also far less marked than one would expect from a consideratio of the postmortem findings As summarized by Chun [o]

> The physical signs in the lungs are often slight even in cases well advanced in the disease On percussion dulness is often absent and the vocal fremitus and resonance unchanged In a small proportion of cases however localized areas of dulness may be distinguished On auscultation râles are frequently not heard except before death When present early in the disease they are usually of the fine variety Numerous rales are heard late in the disease due to the oedematous condition of the lungs Feeble respiratory sounds or pure tubular respiration over small areas are common

While it is not uncommon to find a dry pleuritic rub present in pneumonic plague patients, instances where the presence of a fluid pleuritic exudate could be ascertained through physical examination seem to be exceptional In one such case, described by Durand & Conseil,[37] marked dullness was found over the right side of the chest At autopsy about 500 g of a slightly turbid fluid which contained fairly numerous plague bacilli were found in the right pleural cavity besides a fibrinous exudate covering the visceral and parietal pleurae

The symptoms and signs generally found in patients suffering from primary pneumonic plague are identical with those met with in other severe forms of the disease Signs of rapidly progressing heart failure and usually also nervous disturbances are marked Loss of co ordination of the voluntary muscles, which tended to render the gait of the patients unsteady, was often observed in the Manchurian epidemics

It is of great importance to realize that even in the group of primary pneumonic plague cases called typical for the sake of classification marked variations of the clinical picture are found

The following observations deserve notice in this connexion

(a) *Duration of illness* Though as noted before, pneumonic plague patients not or inadequately treated, usually survive not longer than a few (2 4) days a number of instances of longer survival have been recorded With rare exceptions these longer illnesses did not exceed 9 10 days but it is noteworthy that the serum treated patients observed by Lhuerre & Leger[113] and Uriarte et al,[197] respectively both survived for about a month

(b) *Atypical sputum* Several observers drew attention to the fact that sometimes the sputum of pneumonic plague patients was of a yellowish or rusty colour similar to that found in croupous pneumonia As a rule, the sputum did not long retain such an aspect but occasionally a rusty expectoration continued throughout the illness In exceptional cases the sputum though containing numerous *P pestis* remained salivary or mucous throughout the disease (Osborn & Chervenzoff, quoted by Wu Lien teh[16])

Wagle & Bedarkar [*] stated that in their experience of pneumonic plague patients with lobar involvement the sputum has been found stringy in character, often rusty or mixed with blood and difficult to expectorate.

Similar findings seem to have been made also by Lewin et al.[111] in an atypical case of pneumonic plague with lobar involvement.

A few instances are on record where pneumonic plague patients had a profuse haemoptysis so that the presence of tuberculosis was thought of (Wu Lien-teh [16], Goldstein).

Wu Lien-teh [16] adduced evidence to show that the sputum of pneumonic plague patients tended to lose its characteristic aspects when the sufferers recovered. In exceptional cases the non-characteristic sputum of convalescents continued to harbour *P. pestis*, in one instance observed by Gotschlich [8] up to the 41st day after onset of the illness.

(c) *Frequency of lobar pneumonia.* While, as noted above, generally speaking lobar involvement of the lungs seems to be less frequent in pneumonic plague patients than lobular foci, Hennessey [62] stressed the fact that 14 of the 19 cases of primary pneumonic plague seen since 1933 at postmortem examinations in Kampala, Uganda, had shown a lobar type and that in those cases septicaemia was most uncommon. It appeared also that—evidently in contrast to previous observations—a vast majority of the sufferers seen clinically showed lobar pneumonia. Hennessey argued that this change in type was due to a reduction of the virulence of the *P. pestis* strains in Kampala and maintained that

[*] This conclusion is supported by the protracted course of the illness in some cases, durations of 7 to 9 days being occasionally encountered, and by the fall in the plague death rate in this district since 1935 (712 deaths during the period 1936-39 as against 2,458 deaths during the previous four-year period).

Wright [1], dealing in 1943 with the recent plague situation in Nairobi, Kenya, also pointed to an increased frequency of lobar involvement of the lungs in pneumonic plague victims. He maintained that such cases "perhaps are a form that becomes prevalent after the epidemic has begun to wane. It may perhaps be that, as has been shown to be possible experimentally, the organism may become particularly prone to affect the lung."

Interesting as the contentions of Hennessey and Wright are, they are not in accord with the observations made at the end of pneumonic plague outbreaks in China.

(d) *Spontaneous recovery.* While even in the past occasional success was obtained when patients suffering from primary pneumonic plague were treated with plague serum or—more recently—with sulfonamides, and nowadays spectacular results have been obtained with the aid of antibiotics, instances of recovery of such patients who had not been specifically treated are few and far between. However, a few cases of this kind have

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been recorded by Wu Lien teh [16] and more recently by Estrade [39] and Girard [60]. A further patient, mentioned by Williams,[209] who showed signs of pneumonia with delayed resolution and *P. pestis* in his uncharacteristic sputum but eventually recovered, was treated with Suramin (a complex sulfonated urea derivative known under various trade names and much used in the treatment of African trypanosomiasis).

The evidence for the existence of mild cases of pneumonic plague is not convincing. No instances of this kind were observed during the Manchurian epidemics of 1910-11 and 1920-21. An individual admitted during the 1921 outbreak at Vladivostock with a history of two weeks' headache and slight cough showed *P. pestis* in his sputum. However, as pointed out by Wu Lien teh,[16] only smear examinations seem to have been made and, moreover, it was doubtful whether this man was a plague carrier or was suffering from minor plague. It should be noted in this connexion that nine instances could be found in the available literature in which the presence of virulent *P. pestis* was confirmed in the sputum or fauces of apparently healthy individuals who had been in contact with pneumonic plague patients (Wu Lien teh,[216] Girard,[61] Suarez,[189] Tieh et al.[19]).

It is important to add that occasionally, in pneumonic as well as in bubonic plague, the apparent absence of serious signs may be deceptive, the sufferers soon afterwards succumbing. For instance, Raynaud (quoted by Wu Lien teh [216]) referred to one patient who was found attending his customers and was able to stand while being auscultated, yet died the next morning.

Slight lung involvement

Practically all workers who saw comparatively large numbers of pneumonic plague patients observed that a minority of the sufferers, though showing serious general symptoms and signs, had but slight or even almost no cough and expectoration. There can be no doubt that a close relation exists between these clinical findings and the presence of slight pneumonic changes found at autopsy in a minority of primary pneumonic plague victims.

In some of the patients with apparently slight lung involvement observed by the present writer in China, spontaneous cough was practically absent, so that at first glance these sufferers, who were in a serious general condition, seemed to be victims of "septicaemic" rather than of pneumonic plague. However, they produced typical blood-stained sputum when urged to cough and expectorate.

Similar cases were probably seen by Gale [53] in South Africa. He stated that cough was present in nearly all his patients, though it was generally very slight and productive of little, if any, sputum. However, some patients, urged to do so, produced sputum from the lungs much greater in

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amount than they had done when left to themselves Gale added that physical examination revealed signs of small basal patches of consolidation in several cases which had practically no cough and no sputum whereas in others with more cough (but scanty sputum) there were practically no pathological signs in the chest In Gale s opinion these patients all but one of whom died,

> " seemed to succumb to toxaemia possibly septicaemia which *inter alia* robbed them of the normal vigour of the cough reflex Thus failing to produce sputum in any great quantity they were not as infective as pneumonic patients usually are "

Interesting as this hypothesis is it cannot be considered as generally valid because patients with frank primary pneumonic plague, through often showing signs of marked toxaemia usually continue to cough and expectorate freely until they die

Cases probably similar to those of Gale were observed recently by Seal [171] in Calcutta He noted that though all his patients showed more or less marked signs of lung involvement cough was mostly non productive This seemed the main reason why many of the contacts escaped infection

While the observations recorded above leave no doubt as to the occurrence of cases with slight pneumonic manifestations no sharp lines of demarcation seem to separate this group from that of frank pneumonic plague on the one hand and from the type without lung consolidation on the other hand

Absence of lung consolidation

As summarized by Wu Lien teh [16] the occurrence of cases without cough and expectoration had been recorded by some earlier observers of pneumonic plague epidemics and the occasional absence of lung consolidation had been noted at the autopsy of victims of this form of the disease However not much attention was paid to such observations until Wu Lien teh et al [18] finding instances of this kind to be apparently frequent at the end of the 1921 outbreak at Harbin Manchuria pointed to their epidemiological importance

Girard [57] whose investigations have contributed much to the knowledge of pulmonary plague concluded in 1929 that

> " these abnormal forms develop like septicaemic plague cases However they are preceded by a closed pulmonary stage from which the organism passes into the blood stream but never appears in the expectoration since we could not detect it there by guinea pig experiments The presence of plague bacilli in lung smears fewer than in typical pneumonic forms but more numerous than in liver smears (where they are sometimes altogether absent) testifies that the lungs really are involved These abnormal forms are

therefore pulmonary in their etiology (direct contagion) but septicaemic in their evolution *f*

An excellent clinical description of this pulmonary form of plague was given by Christie [19] in a report, rendered after the 1910 1 Manchurian epidemic wherein he stated that

> about 10 per cent of all cases in this epidemic seem to have been what is called septicaemic—that is to say without any pneumonic manifestation The attack is sudden and death rapid nervous symptoms are prominent there is giddiness and staggering the patient often falling by the roadside great prostration is also present and the patient soon passes into a comatose condition The face after death is very dark Few of this type of the disease have died in plague hospitals as the course of the disease is too rapid

Rapid death of patients who though infected through contact with pneumonic plague sufferers, showed signs of a generalized infection but not of pneumonia, was noted by some other observers as well For instance, Delbreil, [1] apparently referring to six such patients, stated that they died in 2 3 days, whereas the sufferers showing the usual features of pneumonic plague died after 2 6 days

However patients suffering from ' pulmonary plague did not invariably succumb rapidly Two such sufferers, observed by Girard [57] and by Wright [215] respectively, survived for four days That in exceptional cases pulmonary plague patients might survive even longer, seems to be suggested by an observation of Goldstein [7] the man in question was admitted with a history of four days illness on 8 August 1941 and, having shown no physical signs of pneumonia and having produced no sputum died on 11 August Fever was high but (as in some other plague patients seen at the time) the pulse rate was low so that a tentative diagnosis of typhoid was made However, as Goldstein noted without giving details, plague pneumonia was found to be present at autopsy

In Girard s [57] opinion the duration of illness in such cases is dependent upon the time at which septicaemia appeared if this develops rapidly, death occurs quickly, if more slowly, the disease may last four days *g*

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

As has been emphasized earlier, the diagnosis of plague must rest on the results of laboratory examinations rather than on clinical findings except when well marked cases are dealt with during fully confirmed

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f " Les formes anormales dont le nombre est assez élevé évoluent comme une peste septicémique Mais elles sont précédées d un stade pulmonaire fermé d ou le germe ne s extériorise que pour passer dans le sang tand s qu il n apparait jamais dans les crachats puisque nous n avons pu l y déceler par inoculation au cobaye La présence de bacilles pesteux dans les frottis de poumon en proport on mo ndre que dans les formes typiques mais beaucoup plus forte que dans les frottis de foie ou on n en vo t quelquefois pas temoigne de l atteinte réelle du poumon Ces formes anormales sont donc b en pulmonaires par leur étiol gie *(contagion directe) mais septicém ques par leur évolution*

g La durée de la malad e est alors subordonnée à l apparition de la sept cémie celle-ci est-elle précoce la mort survient tres vite est elle plus tardive la malad e peut durer 4 jours

outbreaks However a clinical prima facie diagnosis always important from the viewpoint of plague prevention is now also of utmost therapeutical importance because particularly if sulfonamides only are available optimal results will be obtained only if treatment is started as soon as the presence of the disease is assumed on clinical grounds To wait for laboratory confirmation might particularly under rural conditions render futile attempts to save the patients It is unfortunate therefore, that the presumptive clinical diagnosis of plague is beset with some difficulties

It would seem at first glance that the history of the illness given by the patients themselves or by their families should be of great value in arriving at a presumptive diagnosis of plague both by showing that the sufferers fell suddenly ill a short time ago and by referring to characteristic signs such as the presence of painful buboes or the expectoration of blood stained sputum Ample experience has shown however that the patients themselves and their families often go to almost incredible lengths to mislead the medical staff It is essential therefore to insist in any case where there is reason to suspect plague regardless of the history given upon a thorough examination of the patients including an inspection of all regions of the body where buboes might be present and whenever pneumonic plague might come into question to insist that the patients cough and expectorate

As shown by many experiences the danger of misdiagnosing even well marked plague cases is great in areas where the medical workers are unfamiliar with this disease This holds true not only of regions where plague is altogether absent but also of localities where the infection is present among wild rodents but rarely spreads to human beings The danger to which the latter situation might easily lead is well illustrated by the recent appeal of Link[114] to the physicians in the western States of the USA to regard all cases of inguinal and axillary adenopathy with a high index of suspicion and institute immediate treatment with streptomycin and sulfadiazine

As noted before the medical workers in plague areas may be prone on the other hand to class any patient with enlarged lymph nodes as suffering from plague It has been urged therefore that in all such cases adequate laboratory investigations be made

Earlier emphasis has been laid upon the fact that even in severely affected plague patients general symptoms and signs which are usually present may be conspicuous by their absence Thus the sufferers though fatally infected or even not far from death may not only feel but even seem quite well Fever may be low or absent and the pulse rate may be normal or in exceptional cases there may even be a bradycardia suggestive of typhoid in cases where localized signs of plague infection remain absent Generally speaking the sole presence of general symptoms and signs renders a clinical differentiation of plague from other acute febrile diseases particularly from pernicious malaria difficult

A differentiation of plague from infections caused by pseudotuberculosis bacilli or other members of the genus *Pasteurella* is not as difficult as it might seem at first glance Instances of human infection by pasteurellae other than *P pestis* are rare, invariably remain solitary and so far have been recorded in plague free rather than plague affected areas Moreover, as a rule, the clinical picture produced by these infective agents is markedly different from that in plague

Infection with *P pseudotuberculosis* produces in man almost always a typhoid like syndrome with icterus, a sign usually exceptional in plague (Dujardin Beaumetz [31] Topping et al [173] Hassig et al [78])

Infections due to other pasteurellae according to Tricot & Gauthier [194] never produce a haemorrhagic septicaemia but result in purulent pleurisy, meningitis, myositis, urinary infections, or conjunctivitis Some instances have also been recorded where the scratches or bites of cats, or the bites of dogs, produced local lesions due to pasteurellae

It will be seen that serious differential diagnostic difficulties would be likely to arise only in cases of primary conjunctivitis or meningitis As noted before instances of primary conjunctival plague or apparently primary plague meningitis are rather rare

Though the ulceroglandular, oculoglandular, and glandular types of tularaemia (Francis [5]) might resemble plague, a differential diagnosis on clinical and epidemiological grounds or through laboratory examinations is not difficult As summarized by Wilson & Miles [210]

> In the differential diagnosis [of tularaemia] from plague account should be taken of the absence of pus at the site of infection the greater variability in size of the granules *in the spleen the rarity with which the lungs are affected* and the failure of the organism to develop on ordinary media

Well-Marked Bubonic Plague

A clinical prima facie diagnosis of well developed ' typical bubonic plague is easy because as has been described earlier, the symptoms and signs present in such cases particularly the manifestations in the primarily affected lymph nodes are rather characteristic

In contrast with most other conditions which produce an acute inflammation of peripheral lymph nodes reactions at the portal of entry of the infection are usually not manifest in bubonic plague Moreover if present the appearance of these local processes is as a rule quite characteristic This holds true not only in the cases where carbuncles develop but also if pus filled vesicles form at the site of invasion because their inconspicuousness as contrasted with the marked reactions evolving in the regional lymph nodes is rather peculiar Plague bacilli though sometimes scanty may invariably be demonstrated in the pus of such vesicles

Besides usually leading to marked reactions at the site of infection the manifestations produced by an invasion of other infective agents in the regional lymph nodes and in the system in general are as a rule less stormy and less rapidly progressive than those caused by *P pestis* Commonly, therefore no differential diagnostic difficulties arise in cases of well marked and fully developed bubonic plague However, should doubts exist puncture of the affected lymph nodes should be resorted to, carried out preferably according to the technique recommended by Girard,[9] which renders it easy to use the puncture material for cultivation and/or animal experiments As noted earlier, cultivation of the material on plain media is essential in plague areas where the simultaneous presence of tularaemia might cause differential diagnostic difficulties

It is important to add that according to what has been discussed in chapter 5 bubo puncture though sometimes necessary is by no means an ideal method of confirming the diagnosis of bubonic plague It should be replaced as much as possible by blood cultivation or by serodiagnostic procedures Adequate examinations of the blood are particularly valuable because it is of great prognostic and therapeutic importance to establish whether or not bacteraemia is present

As stated before it is often difficult to decide whether or not secondary pneumonic foci have developed in bubonic plague patients Goldstein[7] recommended the method of lung puncture to ascertain the presence of lung consolidation in patients with bubonic or ill defined pneumonic plague and described his technique thus

> Choosing preferably a site where there appeared to be some dullness on percussion I inserted a needle in several directions and made a smear from the fluid obtained by aspiration "

Even if it were possible to consider this method as safe its reliability seems questionable It might give false positives in cases where pneumonic foci are absent but bacteraemia is marked On the other hand as proved by an observation of Goldstein himself it is bound to give false negatives in cases where instead of the consolidated non-consolidated parts of the lungs have been punctured It would be preferable therefore to resort in such cases to x ray examinations of the lungs which as will be shown later have given most satisfactory results in cases of primary pneumonic plague

In the usually infrequent cases of tonsillar plague as well as in the rare cases of primary eye plague typical buboes as a rule develop in the regional lymph nodes thus facilitating a presumptive clinical diagnosis In view of the fact however that an invasion of the faucial mucous membranes or of the conjunctivae by other micro organisms may produce syndromes similar to those caused by *P pestis* thorough laboratory examinations are indicated whenever the presence of these forms of plague is assumed This

is particularly important in the case of tonsillar plague the clinical appearances of which may be quite similar to those produced by the diphtheria bacillus

Exhaustive laboratory examinations are also indicated in the case where the presence of a primary plague meningitis is assumed, particularly because morbid processes of such a nature may be produced by pasteurellae other than *P pestis*

Pestis Minor

As has been described above, the clinical findings in pestis minor are apt to be so ambiguous as to render a definite diagnosis impossible It is essential, therefore to confirm the presence of plague through laboratory examinations Puncture of the affected lymph nodes, since it causes little discomfort to the patients, may be freely used for this purpose Unless the condition of the patients takes an unexpected turn for the worse, it is legitimate to await the results of laboratory tests before commencing specific treatment

Fulminant Type of Bubonic Plague

The difficulties of arriving at a presumptive diagnosis of what is commonly called ' primary septicaemic ' plague are considerable but should not be overrated chiefly because

(1) almost invariably, cases of this type of the disease do not appear independently but in association with bubonic plague cases, and

(2) even a short period of continued observation shows a rapid deterioration of the condition of the patients which is quite uncommon in most of the diseases with which ' septicaemic plague might be confused at first glance

The present writer for one would not hesitate, therefore, to say that apart from incipient pneumonic plague and from pulmonary " plague, malaria—which is often endemic in the areas where plague exists—is the only disease really difficult to differentiate from septicaemic ' plague

It might seem at first glance that examination of suitably stained blood smears for the presence of malaria parasites ought to be an easy and rapid means of differentiating between this disease and plague However, it is often difficult to carry out such examinations rapidly enough under rural conditions Far more important still for various reasons it is quite often difficult, and sometimes even impossible to prove the presence of parasites in the blood of patients suffering from the pernicious type of malaria which causes the main differential diagnostic difficulties Moreover, as confirmed by observations like that of Le Gall et al [103] 'septicaemic

plague may appear in patients who suffer from manifest malaria Signs of past malaria infection in patients developing septicaemic plague may also confound the issue Thus Yenikomshian[221] recorded the case of a 12 year old girl seen in a comatose condition who because she had come from a malarial district had had malaria in the past, and showed an enlarged spleen seemed at first glance to suffer from pernicious malaria However, the presence of septicaemic plague was proved through cultivation of the blood and also through spleen puncture performed after death

As confirmed by observations of the present writer, an adequately prolonged examination of the blood smears stained for the purpose of detecting malaria parasites occasionally reveals the presence of *P pestis* even in incipient septicaemic plague Since, however such positive findings are rather rare and since, moreover, the apparent absence of malaria parasites from the smears is not fully conclusive, a preliminary decision on whether septicaemic plague or malaria is present has quite frequently to be reached on clinical grounds Pending further observation of the patients or until the results of repeated smear examinations or even of blood cultivation have become available it is sometimes impossible to form a definite opinion Whether it is justifiable under such conditions to begin plague treatment immediately seems debatable As far as the present writer can judge one ought to initiate such treatment under all circumstances as soon as there is reason to suspect the presence of fulminant plague

Primary Pneumonic Plague

Incipience

As will be gathered from the description of the clinical features of pneumonic plague given earlier only general signs of serious illness develop during the initial stage of primary pneumonic plague which lasts 20 24 hours The difficulties of arriving at a diagnosis during this first stage of the disease are therefore similar to those met with in the so-called primary septicaemic" form of plague However the fact that primary pneumonic plague affects exclusively persons who have had recent contact with patients suffering from plague with lung involvement is of utmost diagnostic importance Indeed it is a priori likely that any person who falls ill soon after such contact and shows fever and other general signs suggestive of the presence of an infectious disease suffers from incipient pneumonic plague

Since as has been stated before smear examination of the sputum or saliva carried out during this stage mostly yields inconclusive or even negative results until quite recently a prima facie diagnosis of incipient

pneumonic plague had to be made on clinical grounds. Indeed, one might almost say that one could merely suspect but *not really diagnose the evolution of this disease*.

However, *recently* Estrade,[41] making a series of x-ray examinations in the case of a pneumonic plague patient whom he was able to cure, found with the aid of this method evidence of the formation of pneumonic foci quite early in the disease. In his opinion, *therefore*, "*the importance of the x-ray picture at the time of the first rise in temperature leads one to think that* systematic x-ray examinations of persons particularly exposed to contagion could be a means of diagnosing incipient pulmonary plague".[h]

As can be gathered from recent publications by Mercier[130] and by Mercier & MacCrumb,[131] *this postulation has been confirmed*. *The* value of this new diagnostic method for the treatment and control of pneumonic plague as well as for further studies on the pathogenesis and pathology of this disease can hardly be overrated.

Manifest stage

Owing to the presence of general symptoms and signs of a serious and *rapidly progressing illness, associated with often comparatively insignificant* signs of lung involvement and the expectoration of a peculiar blood-stained sputum, it is easy to make a presumptive diagnosis during the manifest stage of primary pneumonic plague. Since the sputum teems with plague bacilli, it is also not at all difficult to confirm this diagnosis through *laboratory examinations*. *In view of the fact that, in rare cases, an invasion* of the lungs by micro-organisms other than the plague bacillus produced morbid features more or less similar to primary pneumonic plague, adequate culture methods and/or animal experiments should be resorted to in addition to smear examinations. However, one might be less rigid when dealing with quite typical cases during a fully established pneumonic plague epidemic.

If adequate laboratory *tests are applied, there is also no difficulty in* arriving at a correct diagnosis in cases where unusual features, such as a rusty or otherwise atypical aspect of the sputum or lobar instead of the usual lobular type of lung involvement, are present. It is true that sometimes cases are met which at first glance resemble croupous pneumonia. However, the *rapid deterioration* of the general condition of the patients becoming manifest in such atypical cases as well as in the usual type of pneumonic plague ought to suggest the *necessity for thorough laboratory* investigations. As has been said before, plague cases will hardly ever be overlooked if the possibility of their occurrence is kept in mind.

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[h] "L'importance de l'image radiographique dès la poussée initiale de température permet de penser que la radiographie systématique des personnes qui ont été particulièrement exposées à la contagion pourrait, le cas échéant, fournir *une méthode de diagnostic précoce de la peste pulmonaire*."

As previously noted, probably owing to the presence of slight pneumonic changes, spontaneous cough and expectoration may be practically absent in some patients suffering from primary pneumonic plague. In order not to overlook the presence of this form of the disease one should urge all plague patients with features suggestive of a generalized infection to cough and to expectorate. No doubt x-ray examination would be most helpful in such cases.

"Pulmonary" Plague

Though the information available in regard to the clinical aspects of the form of lung plague in which no pneumonic foci develop is scanty, there can be no doubt that the clinical manifestations present in such cases correspond to those met with in patients suffering from what is called primary septicaemic plague. It follows that the considerations and methods found useful for the diagnosis of primary septicaemic plague will also prove valuable for the recognition of the "pulmonary" form. Comparatively speaking, the latter is less difficult to diagnose, because it develops invariably in persons who have had recent contact with pneumonic plague patients and usually, or perhaps even always, appears only after the occurrence of typical cases of primary pneumonic plague.

TREATMENT

Though *attempts to cure plague may be said to date back as far as* the history of this disease, the first milestone on the road to real success was reached only when in 1896 Yersin[illegible] began to use a therapeutic serum produced through immunization of horses with *P. pestis*. From that time onwards until recently administration of sera prepared either according to Yersin's original procedure or in other ways remained the only method which, because it gave a modicum of success in the case of the bubonic form, was used on a large scale for plague treatment. The use of sulfonamides, commenced about 14 years ago, greatly improved the therapeutic results in bubonic plague. Quite recently a most outstanding success in the treatment of all forms of the disease has been attained with the aid of streptomycin and some other antibiotics. Indeed the results obtained with these therapeutic agents have been so consistently good, even in most severely affected patients, as to relegate the sulfonamides to the second rank in the treatment of bubonic plague and, as will be discussed later, to render rather questionable the necessity of using the specific sera any more.

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" Pulmonary " Plague

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TREATMENT

Though attempts to cure plague may be said to date back as far as the history of this disease the first milestone on the road to real success was reached only when in 1896 Yersin[2] began to use a therapeutic serum produced through immunization of horses with *P pestis* From that time onwards until recently administration of sera prepared either according to Yersin s original procedure or in other ways remained the only method which because it gave a modicum of success in the case of the bubonic form was used on a large scale for plague treatment The use of sulfonamides commenced about 14 years ago greatly improved the therapeutic results in bubonic plague Quite recently a most out standing success in the treatment of all forms of the disease has been attained with the aid of streptomycin and some other antibiotics Indeed the results obtained with these therapeutic agents have been so con sistently good even in most severely affected patients as to relegate the sulfonamides to the second rank in the treatment of bubonic plague and as will be discussed later to render rather questionable the necessity of using the specific sera any more

Serotherapy

It is difficult to deal comprehensively with the results obtained with serum treatment of plague, not so much because an enormous literature deals with this subject, but mainly because the recorded results are often not comparable The reasons for this incomparability are as follows

1 While the observations of some of the workers were based upon sufficiently large samples to show statistically significant results others reported upon limited numbers of treatments or merely upon a few cases

2 The serum dosages used by the different workers varied widely and sometimes insufficient doses were used

3 Marked differences may exist in the efficacy of sera prepared by different methods

4 The loss in potency which sera undergo in storage may vitiate the results when different lots of the same serum or different sera prepared at different times are compared

5 Ample observations, recently confirmed by the exact tests of Sokhey et al [181] referred to below, have shown that the results of serotherapy, as well as those of other methods of specific plague treatment, depend largely upon the absence or presence of bacteraemia in the subjects treated Hence the results obtained in countries or in outbreaks where a benign type of plague prevailed are not comparable with those obtained during plague manifestations characterized by a prevalence of serious types of the disease frequently resulting in bacteraemia One must agree with Sokhey and his colleagues that only the results obtained in patients who show evidence of bacteraemia on admission form standards for assessing the efficacy of different methods of plague treatment Lack of indications regarding the presence or absence of bacteraemia in the treated patients often renders it impossible properly to evaluate and compare the older records on the serum treatment of plague

It seems indicated, therefore that one should assess the results of plague serotherapy in a general manner instead of scrutinizing the records of the individual workers in detail

General agreement exists that it was but rarely possible to save patients suffering from primary pneumonic plague through serum administration alone In addition to some more or less creditable instances of this kind recorded by Wu Lien teh [16] in 1926, recent successes in the sero therapy of pneumonic plague have been reported by De Moura et al [140] (three cures in patients treated early in the disease with average doses of 150 230 ml of plague serum administered during two days partly intra venously and partly intramuscularly) Gale [53] (bacteriologically uncon firmed case of a girl, who had been vaccinated against plague five or six

days before she had had contact with a plague patient and who was given 200 ml of plague serum on the fourth day of illness), and Clark & Goldberg[21] (an old woman who had been given 1 ml of live avirulent vaccine and 50 ml of plague serum prophylactically and received when falling ill with bacteriologically confirmed pneumonic plague a total of 400-500 ml of plague serum intramuscularly and subcutaneously)

Only two instances of recovery in serum treated patients suffering from primary septicaemic plague could be found in the available recent literature and it has to be noted that in the case reported by Le Gall et al[103] treatment consisted of the production of a fixation abscess as well as of the administration of 320 ml of serum whereas the patient referred to by Erzin & Payzin[38] seems to have been treated with sulfonamides as well as with plague serum

For the reasons stated above the results obtained by the different workers when using serotherapy in bubonic plague cases and consequently the opinions they formed regarding the value of this mode of treatment varied greatly While the initial results of Yersin[2] and also those with Lustig s serum[1] were most favourable and equally good experiences were recorded by a few later workers (e g by Joltrain[91] during the mild outbreak of plague at Paris in 1920) some observers altogether denied the value of plague serotherapy and many others considered it effective only when administered early in the disease Choksy (quoted by Simpson[17]) who had exceptionally wide experience in the treatment of plague at Bombay shared the latter belief stating that

> Should those who are conversant with the application of the serum in plague be entrusted with 100 persons provided the cases are not septicaemic they would be able to bring round at least 60 if not more by the use either of Lustig s or Roux s serum of the strength that has been used in Bombay during the last two epidemics "

As summarized by Dieudonne & Otto[4] the Indian Plague Commission considered that both Lustig s and Yersin s serum exerted some influence on the clinical course of plague but that the effect was by no means as marked as in the case of diphtheria serum Nevertheless in the opinion of the Commission attempts to produce a potent serum ought to be continued since serotherapy was the only method giving some hope for an effective treatment of plague

As stated in chapter 3 some new types of plague sera have recently become available two of which—the serum produced with the aid of the avirulent EV strain in Madagascar and the new Haffkine Institute serum—have been used on a fairly large scale with quite satisfactory results Girard[6] stated in this connexion that with the aid of the new

[1] The [illegible]

Madagascar serum it was possible to cure 60%-65% of the bubonic plague patients, and similar results were also recorded by Le Gall [10]

As noted before, in their studies on plague treatment Sokhey et al [184] gave separate consideration to the results obtained in all cases and to those in patients who showed evidence of bacteraemia on admission They described the simple method they used for this purpose as follows [18]

On admission the diagnosis was first made on clinical grounds At the same time before any treatment was given 0 5 cc of blood was drawn from a vein and planted in equal quantities on two agar slopes This was done to determine the presence or absence of plague septicaemia at the commencement of treatment If the cultures remained sterile after two days incubation at room temperature and there was still doubt about the diagnosis the bubo was punctured with a syringe and the fluid cultured on agar slopes to *confirm the diagnosis*

The results obtained by Sokhey and his colleagues [184] from 1941 to 1948 when treating bubonic patients with Haffkine serum may be summarized thus

	Number of cases	*Number of deaths*	*Mortality ( )*
All cases	157	37	23 5
Cases with plague bacteraemia on *admission*	*71*	*36*	*50 7*

There was only one death among the 86 patients who showed no bacteraemia at the commencement of treatment as compared with 36 deaths among the 71 sufferers showing evidence of bacteraemia on admission

It has already been stated that the serum dosages used by the different workers varied considerably Some utilized doses which cannot be con *sidered as sufficient, while a few particularly* when attempting to save pneumonic plague patients administered enormous amounts of serum

Sokhey et al [184] recommended administering 40 ml of serum to bubonic plague patients on the first day of treatment giving half this amount intra venously on admission and the other half subcutaneously six hours later and giving the same amounts in a similar manner on the following day While in their experience no further treatment was required in mild cases it was necessary to administer further doses to severely affected patients usually for five days

As summarized by Le Gall [10] workers in Madagascar used to give the serum prepared with the aid of the EV strain in average daily doses of 40 60 ml for five days

Using different sera some recent workers recommended the use of higher daily doses of up to 100 ml for adults and 50 ml for children (Lobo & Silvetti [115]) However when potent sera are available daily doses of 40 60 ml may be said to suffice in general

Sulfonamide Treatment

The history of plague treatment with sulfonamides may be said to go back to the year 1937, when Buttle et al[11] proved experimentally that sulfanilamide exerted some action on *P pseudotuberculosis* and *P septica* Working with *P avicida* Levaditi & Reinic[108] (1938) obtained identical results

Carman[14] and Vine[03] recorded in 1938 that they had each been able to save three bubonic plague patients through intramuscular injections of Prontosil (*p* aminophenylsulfonamide) while van Hoof[8] reported in the same year that in the Belgian Congo two out of three patients treated with sulfanilamide had been cured No details could be elicited regarding the statement of Prado Barrientos[15] that during the 1938 bubonic plague outbreak in the Santa Cruz department of Bolivia he had obtained fairly satisfactory results with emergency chemotherapy

Important experimental evidence of the efficacy of sulfonamides in plague treatment was furnished in 1939 by Schutze[170] Durand[34, 35] and Girard & Girard[69, 70]

As summarized in 1941 in a *British Medical Journal* editorial[8] Schutze found that when sulfapyridine soluseptasine and a diaminodiphenyl sulfone glucose compound were given to mice and rats subcutaneously or by mouth sulfapyridine proved the most efficient drug in both animal species Finding that the plague serum prepared in the Lister Institute with the aid of Otten s avirulent strain gave results comparable to those obtained with sulfapyridine, Schutze suggested that a combination of sulfonamide treatment with serotherapy might be advantageous

Durand[34, 35] feeding mice with daily sulfapyridine doses ranging from 1 25 mg to 2 25 mg per gram of body weight for periods of 6-7 days after plague infection found that the animals so treated resisted at least 10 000 *P pestis* Two further important facts established in the course of these investigations were that (*a*) it was often impossible to demonstrate *P pestis* in sulfapyridine treated mice which succumbed because they had received too high an infecting dose and (*b*) 21 out of 36 sulfapyridine treated mice which had resisted plague when challenged 33 days after the original infection proved to be immune to 2 or even 20 lethal doses of *P pestis*

Girard & Girard[69, 70] also obtained satisfactory results when administering sulfapyridine to mice and guinea pigs even when the treatment had been started at the time of plague infection or in the case of some of the guinea pigs up to two days after infection Like Schutze these two authors pointed to the possibility that combined sulfonamide and serum treatment might improve the therapeutic results

Commenting upon the experiments of Durand and also upon observations made regarding the action of sulfonamides on other micro-organisms

a reviewer stated in the *Bulletin mensuel de l'Office International d'Hygiène Publique*[9] that since test animals succumbing to plague infection after insufficient sulfa medication were often free from *P. pestis* while those surviving infection, because sufficiently treated, were frequently immune, one might assume that

> Dagenan (sulfapyridine) destroys the plague bacillus leaving the circulating toxin and the antigen intact. Since the degree of immunity is in correlation with the comparatively high dose of microbes used for the first infection one could conclude that this immunity is produced solely by the preformed toxin, perhaps also by that which can be freed by bacteriolysis. J

As shown by Girard,[65] sulfapyridine did not protect mice against toxic filtrates of *P. pestis*. The observations of several workers that combined administration of sulfonamides and plague serum was apt to give better results than treatment with either remedy alone seem also compatible with the idea that, in contrast to the serum, the sulfonamides exert no antitoxic action (Girard[65]). Further evidence is also at hand to render it likely that, as a recent editorial[87] puts it, "the sulpha drugs interfered with the growth of the organism *(P. pestis)* but not with the production of the antibodies".

Bubonic plague

Large-scale use of sulfonamides for the treatment of bubonic plague was started during the period 1940-1. The early workers had to rely upon sulfapyridine and sulfathiazole, the efficacy of the latter for plague treatment having been proved experimentally by Sokhey & Dikshit[178] in 1940. Afterwards, however, sulfadiazine and its derivatives were mostly used, not only because these newer drugs were less apt to produce toxic reactions, but also because in the experience of most workers they were more effective for plague treatment than sulfapyridine and sulfathiazole. However, no full agreement has been reached in this respect, Macchiavello[117] for instance, considering sulfathiazole to be theoretically best for the treatment of plague.

General agreement has been reached that, since they exert merely a bacteriostatic action, the sulfonamides are most apt to cure plague patients if treatment is started early in the disease. Even then administration of these drugs, though curbing further progress of the infection, does not as a rule bring about a rapid devolution of the buboes which frequently become suppurated in spite of the treatment.

The superior value of early commenced sulfonamide treatment of bubonic patients has been well demonstrated by the observations made

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J "le Dagénan (sulfapyridine) détruit le bacille pesteux tout en laissant intacts la toxine circulante et l'antigène. Comme le degré d'immunité est en rapport avec la dose de microbe plus élevée employée pour la première injection infectante, on pourrait en conclure que cette immunité est provoquée uniquement par la toxine préformée, peut-être aussi par celle qui pourrait être mise en liberté par une lyse bactérienne."

by Sokhey and his collaborators from 1941 to 1948, which according to their recent summary [184] gave the following results

(a) Cases in which treatment was started before the appearance of bacteraemia

Drug	Number of cases	Number of deaths	Mortality (%)
Sulfapyridine	60	2	3 3
Sulfathiazole	155	9	5 8
Sulfadiazine	62	3	2 5
Sulfamerazine	91	2	2 2
Iodine solution (controls)	74	12	16 2

(b) Patients showing bacteraemia at the commencement of treatment

Drug	Number of cases	Number of deaths	Mortality (%)
Sulfapyridine	62	31	50 0
Sulfathiazole	119	50	42 0
Sulfadiazine	62	13	21 0
Sulfamerazine	22	7	31 8
Iodine solution (controls)	75	68	90 7

It will be noted that sulfadiazine gave fairly satisfactory results even when treatment was started late The number of observations made in this respect with sulfamerazine is too small to permit final conclusions but to judge from the available information this drug was more effective than sulfapyridine and sulfathiazole

Indications regarding the dosages of the drugs used during these trials were given by Sokhey and his collaborators thus [184]

" *Sulphapyridine and Sulphathiazole* 10 to 14 g were given on the first day and on subsequent days 6 g depending upon the severity of the case for a maximum period of 10 days The drug was stopped earlier if the temperature came to normal or the patient showed distinct improvement in general condition To comatose patients or when the drug produced persistent vomiting the drug was given intra muscularly or intravenously

Sulphadiazine An initial dose of 4 g was followed by 2 g four hours later Thereafter 1 g was given every 4 hours till the patient s temperature remained normal for 2 days This dosage maintained a concentration between 10 to 20 mg per 100 cc of blood

Sulphamerazine An initial dose of 4 g was followed by 2 g four hours later Then 1 g was given every 8 hours till the temperature remained normal for 2 days This dose maintained a concentration between 10 to 20 mg per 100 cc of blood

Sulfamethazine (sulfamezathine) has been used for plague treatment in the place of the above mentioned sulfonamides Datt Gupta [23] comparing the effect of this drug with that of sulfadiazine recorded the following results

Drug	Dosage	Cases	Deaths	Mortality (%)
Sulfadiazine	4 g initially then 2 g 4 hourly for 4 7 days (per os)	71	7	9 9
Sulfamezathine	3 g by intravenous injection then 2 g 4 hourly for 4 7 days (per os)	37	4	10 8

As far as these figures go sulfamethazine was about as effective as sulfadiazine but it has to be noted that the latter drug was given solely

by the oral route without an initial intravenous administration of its sodium salt

Sulfamethazine is amply used in Java where even rather small doses averaging 3 g per day have been found satisfactory for the treatment of bubonic plague patients, particularly those who had been previously vaccinated with live avirulent plague bacilli

That generally, previous plague vaccination markedly improved results of sulfonamide treatment in bubonic patients is illustrated the following results recorded by Patel & Rebello [149]

	Treated with sulfonamides attacks	deaths	mortality (%)	Untreated attacks	deaths	mortality (%)
Inoculated	1 586	344	21 6	410	165	40 2
Uninoculated	1 262	575	45 5	723	430	59 4
Total	2 848	919	32 2	1 133	595	52 5

Usually sulfathiazole

Since the efficacy of plague treatment with sulfonamides depends upon the establishment and maintenance of a sufficiently high level of the drugs in the blood (10 mg per 100 ml according to Macchiavello [117] in the case of sulfathiazole, sulfadiazine and sulfamerazine), it is in the opinion of many observers, advantageous to initiate treatment by administering a sufficiently high dose by the intravenous route instead of giving an adequate initial dose by mouth Further, though hand in hand with the improvement of the patients the dosage of the sulfonamides should be gradually reduced, it is essential to continue the treatment even during the first few days of convalescence so as to avoid relapses or complications The recommendations made in these respects by the WHO Expert Committee on Plague at its second session held at Bombay in December 1952,[413] were that

> Early cases of bubonic plague could be effectively treated with sulfonamides using a dosage of about 10 g on the first day followed by smaller doses making a total of at least 50 g during the first week The first administration of sulfonamides in the dose of 2 g should be made intravenously preferably in glucose solution In order to prevent relapses particularly meningeal plague the treatment with sulfonamides should be continued for at least 3 days after the temperature becomes normal

Primary pneumonic plague

Although, as unanimously upheld by all observers cases of primary pneumonic plague are not as a rule amenable to sulfonamide treatment the following instances of exceptional success with this therapy could be found in the available literature

Author	Date	Number treated	Number cured	Remark
Van Hoof [83]	1939	12	2	Treated with sulfonamide
Favarel [43]	1945	?	1?	Child with fever but no pneumonia in whose saliva *P pestis* was found Cured with sulfapyridine

Author	Date	Number treated	Number cured	Remarks
Magrou[122]	1945	1	1	Treated with sulfadiazine
Munter[14]	1945	1	1	Previously vaccinated against plague Treated with sulfadiazine
Roux & Mercier[167]	1946	5	3	Treated with sulfathiazole Two of the recovering patients received also plague serum on the 6th and 8th day of illness respectively while a fixation abscess was produced in the third
Favarel[81]	1947	13	2	Treated with sulfapyridine and thiazomide
Than Aung[191]	1947	1	1	Treated with sulfathiazole
Favarel et al[4]	1948	?	3	Treated with sulfathiazole and thiazomide Two of the patients had been inoculated with live plague vaccine while the third had received 14 g of sulfathiazole prophylactically
Tieh et al[192]	1948	5	3	Treated with sulfadiazine

Note

(1) The patient observed by Plum[153] though considered to have recovered from primary pneumonic plague by some reviewers had actually bubonic plague with secondary lung involvement

(2) Some further instances of recovery from primary pneumonic plague will be discussed later because the patients in question had received plague serum and/or streptomycin in addition to sulfonamides

Combination of serotherapy and sulfonamide treatment

As has been mentioned before some of the early workers studying the efficacy of the sulfonamides in experimental plague considered the advisability of combining treatment with these drugs with the administration of specific serum

Actually almost all workers who gave such combined treatment to bubonic plague patients found the therapeutic results superior to those obtained with either sulfonamides or serum It has to be noted however that the differences found in favour of the combined treatment were not invariably marked and that Sokhey and Wagle[183] though establishing that combined treatment with sulfathiazole and plague serum gave better results than medication with sulfathiazole alone (68% cures as against 58 4%) found that sulfadiazine alone gave still better results (79 1% cures)

Besides the above mentioned statement of Roux and Mercier[167] that they had succeeded in curing two patients with primary pneumonic plague by a combination of sulfathiazole treatment with the late administration

by the oral route without an initial intravenous administration of its sodium salt

Sulfamethazine is amply used in Java where even rather small doses averaging 3 g per day, have been found satisfactory for the treatment of bubonic plague patients, particularly those who had been previously vaccinated with live avirulent plague bacilli

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	Treated with sulfonamides			Untreated		
	attacks	deaths	mortality (%)	attacks	deaths	mortality (%)
Inoculated	1 586	344	21 6	410	165	40 2
Uninoculated	1 262	575	45 5	723	430	59 4
Total	2 848	919	32 2	1 133	595	52 5

* Usually sulfathiazole

Since the efficacy of plague treatment with sulfonamides depends upon the establishment and maintenance of a sufficiently high level of the drugs in the blood (10 mg per 100 ml according to Macchiavello [117] in the case of sulfathiazole, sulfadiazine, and sulfamerazine), it is in the opinion of many observers, advantageous to initiate treatment by administering a sufficiently high dose by the intravenous route instead of giving an adequate initial dose by mouth Further though hand in hand with the improvement of the patients the dosage of the sulfonamides should be gradually reduced, it is essential to continue the treatment even during the first few days of convalescence so as to avoid relapses or complications The recommendations made in these respects by the WHO Expert Committee on Plague at its second session, held at Bombay in December 1952,"[13] were that

Early cases of bubonic plague could be effectively treated with sulfonamides using a dosage of about 10 g on the first day followed by smaller doses making a total of at least 50 g during the first week The first administration of sulfonamides in the dose of 2 g should be made intravenously preferably in glucose solution In order to prevent relapses particularly meningeal plague the treatment with sulfonamides should be continued for at least 3 days after the temperature becomes normal"

Primary pneumonic plague

Although, as unanimously upheld by all observers cases of primary pneumonic plague are not as a rule amenable to sulfonamide treatment the following instances of exceptional success with this therapy could be found in the available literature

Author	Date	Number treated	Number cured	Remarks
Van Hoof [81]	1939	12	2	Treated with sulfonamide
Favarel [43]	1945	?	1?	Child with fever but no pneumonia in whose saliva *P pestis* was found Cured with sulfapyridine

Author	*Date*	*Number treated*	*Number cured*	*Remarks*
Magrou [122]	1945	1	1	Treated with sulfadiazine
Munter [14]	1945	1	1	Previously vaccinated against plague Treated with sulfadiazine
Roux & Mercier [167]	1946	5	3	Treated with sulfathiazole Two of the recovering patients received also plague serum on the 6th and 8th day of illness respectively while a fixation abscess was produced in the third
Favarel [44]	1947	13	2	Treated with sulfapyridine and thiazomide
Than Aung [191]	1947	1	1	Treated with sulfathiazole
Favarel et al [4]	1948	9	3	Treated with sulfathiazole and thiazomide Two of the patients had been inoculated with live plague vaccine while the third had received 14 g of sulfathiazole prophylactically
Tieh et al [192]	1948	5	3	Treated with sulfadiazine

Note

(1) The patient observed by Plum [153] though considered to have recovered from primary pneumonic plague by some reviewers had actually bubonic plague with secondary lung involvement

(2) Some further instances of recovery from primary pneumonic plague will be discussed later because the patients in question had received plague serum and/or streptomycin in addition to sulfonamides

Combination of serotherapy and sulfonamide treatment

As has been mentioned before some of the early workers studying the efficacy of the sulfonamides in experimental plague considered the advisability of combining treatment with these drugs with the administration of specific serum

Actually almost all workers who gave such combined treatment to bubonic plague patients found the therapeutic results superior to those obtained with either sulfonamides or serum It has to be noted however that the differences found in favour of the combined treatment were not invariably marked and that Sokhey and Wagle [183] though establishing that combined treatment with sulfathiazole and plague serum gave better results than medication with sulfathiazole alone (68% cures as against 58 4%) found that sulfadiazine alone gave still better results (79 1% cures)

Besides the above mentioned statement of Roux and Mercier [167] that they had succeeded in curing two patients with primary pneumonic plague by a combination of sulfathiazole treatment with the late administration

by the oral route without an initial intravenous administration of its sodium salt

Sulfamethazine is amply used in Java where even rather small doses averaging 3 g per day, have been found satisfactory for the treatment of bubonic plague patients, particularly those who had been previously vaccinated with live avirulent plague bacilli

That, generally, previous plague vaccination markedly improved the results of sulfonamide treatment in bubonic patients is illustrated by the following results recorded by Patel & Rebello [149]

	Treated with sulfonamides			*Untreated*		
	attacks	*deaths*	*mortality* ( )	*attacks*	*deaths*	*mortality* ( )
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Besides the above mentioned statement of Roux and Mercier [167] that they had succeeded in curing two patients with primary pneumonic plague by a combination of sulfathiazole treatment with the late administration

of plague serum, only two records of recovery of pneumonic patien receiving such combined treatment could be found

(1) The case referred to by Wang [207] was that of a nurse who contracte infection in the isolation hospital at Changteh China, and who was actual treated by the present author with sulfathiazole and also with plagu serum, when her condition became desperate She suffered possibly, bi not certainly, from primary pneumonic plague with an atypically lon evolution

(2) Chang [15] recorded one instance of recovery among eight patient with primary pneumonic plague treated in the isolation hospital at Foochow China with sulfadiazine and plague serum

Antibiotic Treatment

Penicillin

In vitro tests made by Magrou,[121] *as well as animal experiments performec* by Witlin and Wilbar,[211] Gupta et al ,[76] Herbert,[81] and Sokhey & Habbu [17[illegible]] showed that penicillin even if used in high doses, *exerted no, or practicall* no, action on *P pestis* These laboratory findings were confirmed by the unfavourable clinical experiences of observers such as Barreto & Castro,[3] Haddad & Valero,[77] and Link [114] Nevertheless, a few workers continued to use penicillin in plague cases in addition to specifics like sulfonamides or streptomycin in order to forestall or to control complications secondary pneumonia (Videla [200]) or secondary infections In the experience of Macchiavello (personal communication to the *Boletin de la Oficina Sanitaria Panamericana* [5]) penicillin was not efficacious in warding off plague *complications*

Streptomycin

Studies on the action of streptomycin on the plague bacillus in vitro and on plague infected animals were first carried out in 1944 by Meyer and his colleagues (Meyer & Quan [135]) Further laboratory studies by several workers such as Sokhey and collaborators,[179] [181] Hornibrook [84] Sergiyev,[17] Macchiavello,[117] Wayson & *McMahon* [203] *Herbert* [81] Quan et al ,[159] Girard,[66] Favarel [46] Neel [144] and Meyer et al [138] confirmed the early findings of Meyer and his colleagues that in vivo as well as in vitro streptomycin exerted a powerful action on *P pestis*, which appeared to be bactericidal rather than bacteriostatic

In vitro studies carried out in Meyer s laboratory showed according to Meyer & Quan [135]

" that some plague bacilli have the same ability to resist streptomycin as have other types of bacteria It was found that although the ratio of the slightly resistant mutants varied from strain to strain usually only few highly resistant bacilli were present in a total of several hundred billion organisms "

The most outstanding result of the administration of streptomycin to plague infected animals was that pneumonic as well as bubonic affections could be readily cured with the aid of this antibiotic

In the course of the investigations of Meyer and his collaborators the bactericidal action of streptomycin in experimental pneumonic plague was fully demonstrated by comparative bacteriological counts of the entire lung tissue of treated and untreated mice which had been killed at various intervals As stated by Meyer & Quan [13]

"By the 96th hour after infection when all untreated mice had died the lungs and bronchial lymph nodes of treated mice either were sterile or contained only a few thousand plague bacilli in the abscess like patches of pneumonia It was impossible to isolate plague bacilli from lung or lymph nodes 100 hours after treatment with strepto mycin had been initiated

Longer periods of treatment (6 days in the case of intranasal infection and 10 days in animals infected by inhalation) were needed to sterilize the lung lesions of experimentally infected guinea pigs

Girard [66] experimenting with guinea pigs infected with *P pestis* by the intratracheal route was able to cure all 12 animals which had been treated with streptomycin from the 30th or 48th hour after infection whereas the controls died in 3 days He ascribed this splendid result to the fact that the antibiotic exerted a bacteriolytic as well as a bacteriostatic action and added that

" the absence of immunity in the cured animals must be ascribed to this double effect which is extremely rapid such a resistance to a new infection would demonstrate the persistence of the causative organisms in the body which is the rule in sulfonamide treated guinea pigs until they recover [k]

Neel [144] also noted a rapid bactericidal action of streptomycin in intra tracheally infected guinea pigs the animals becoming free from plague bacilli two days after commencement of treatment During this time the pathogenic power of the organisms still harboured in the animal body became first lowered and then lost

Quan et al [158] showed experimentally that in contrast to aureomycin oxytetracycline [l] and chloramphenicol streptomycin did not protect mice against the action of plague toxin This result is in accord with earlier observations of Ramon et al [161] that culture filtrates of *Actinomyces griseus* as well as of *Penicillium notatum* did not materially alter the toxicity of this toxin for mice

Streptomycin seems to have been first used for the treatment of human plague by Videla [90] in December 1946 Even though only one of the five patients he treated with this antibiotic suffered from typical bubonic

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[k] C est à ce double effet qui est extrêmement rapide qu il faut attribuer l absence d immunité chez les animaux guéris cette résistance à une nouvelle infection témoignerait de la persistance du germe spécifique dans l organisme ce qui est la règle chez les cobayes traités par les sulfamides lorsqu ils guérissent

[l] Oxytetracycline is the non proprietary name for Terramycin "

plague, while three of the others seem to have had "septicaemic" plague and one showed meningeal involvement, all were cured

Further results obtained with streptomycin in the treatment of plague may be summarized as follows

(a) Bubonic plague

Author or reference	*Date*	*Number treated*	*Number cured*	*Remarks*
Datt Gupta [23]	1948	24	20 (83 3%)	"Only severe cases were selected for streptomycin treatment and two died within 12 hours of admission
Haddad & Valero[77] and Pollock [155]	1948	3	3	The patients who had not responded to treatment with sulfonamides and penicillin rapidly improved and finally recovered when given streptomycin Buboes were not influenced when streptomycin treatment was started late
Karamchandani & Sundar Rao [86]	1948	5	5	Moribund patients four of whom had not responded to previous treatment with sulfathiazole and/or serum
Wagle [204]	1948	32	29 (90 6%)	All these patients had bacteraemia when treatment was started
Chen [18]	1949	3	3	Severely affected patients who had not responded to sulfadiazine Buboes little influenced by streptomycin treatment had to be incised in two of the cases
Estrade [40]	1949	1	1	—
Karamchandi & Sundar Rao [87]	1949	15	12 (80 0 %)	All patients apparently moribund The three fatal cases had been admitted on the third day of illness and died within a few hours
Robic & Favarel [164]	1949	5	5	Received either only streptomycin or also plague serum and sulfonamides
Ghosh [55]	1950	155	149 (96 1 %)	Apparently all treated with streptomycin and sulfadiazine
Link [114]	1950	3	3	Received sulfadiazine penicillin and in two cases also aureomycin in addition to streptomycin Two patients treated only with penicillin died
Soulage et al [186]	1950	14	14 (100%)	Treatment with streptomycin only (4 cases) or with streptomycin and afterwards with sulfonamides (8 cases) The two remaining patients had not responded to treatment with plague serum and sulfonamides but were cured with streptomycin
Archives de l Institut Pasteur de Tananarive	1951 (p 44)	13	11 (84 6%)	With the exception of two also treated with sulfonamides
Archives de l Institut Pasteur de Tananarive	1952 (p 66)	31	31 (100 %)	With the exception of six also treated with sulfonamides

Author or reference	Date	Number treated	Number cured	Remarks
Rao [16]	1952	316*	304 (96.2%)	The patients were treated with streptomycin and sulfonamides except in a few serious cases not more than 2 g of streptomycin were used per patient
Sokhey et al [184]	1952	148	142 (95.8%)	Four of the deaths occurred in the 37 patients showing bacteraemia at the commencement of treatment
Dubey [23]	1953	18	18 (100%)	14 of these patients received only 1 g of streptomycin while four had to be given a second equally large dose after 24 hours Buboes subsided without manifest suppuration

* Sixteen of these patients were said to have suffered from "septicaemic" plague but no separate statistics were given for them

(*b*) *Septicaemic and meningeal plague*

In addition to the observations of Videla [200] and Rao [16] which have been noted above Fain et al [42] reported success when treating two patients suffering from "primary septicaemic" and primary meningeal plague respectively with streptomycin and sulfadiazine

(*c*) *Primary pneumonic plague*

Author	Date	Number treated	Number cured	Remarks
Favarel [4]	1948	1	1	—
Huang et al [85]	1948	1	1	Also treated with sulfonamides and—to prevent secondary infections—with penicillin *P pestis* could be isolated from the sputum of this patient for about a month after onset of illness but soon became avirulent for guinea pigs and mice
Lewin et al [111] **	1948	1	1	Also treated with sulfonamides plague serum and penicillin
Wagle & Bedarkar [20]	1948	5	5	Three of these patients were also treated with plague serum the other two also received sulfamerazine
Estrade [40, 41]	1949 1951	1	1	Sputum though continuing to harbour suspicious bacilli was no longer pathogenic for guinea pigs after first streptomycin dose (0.5 g)
Feng [43]	1949	1	1	Treated also with sulfadiazine plague serum and penicillin
Robic & Favarel [164]	1949	3	3	—
Seal [171]	1949	1	1	—
Bouillat [7]	1951	3	3	—
Guiller & Jospin [7]	1951	5	2	—
Estrade [41]	1951	1	1	—
Mercier [129]	1951	10	9 (90%)	—
Fain et al [4]	1951	1	1	—

** Meyer & Quan referred also to a second pneumonic patient cured in South Africa with streptomycin

Discussing the results obtained in pneumonic plague treatment by Mercier,[129] Girard [68] stressed that one of Mercier's 10 patients after having shown signs of temporary improvement, again became feverish and developing a lung abscess, eventually succumbed In view of the fact that this secondary lung affection was not caused by *P pestis* but was obviously due to a secondary infection, Girard urged that in order to prevent such complications, plague patients should be treated with sulfonamides or penicillin as well as with streptomycin

Considering this postulation, the WHO Expert Committee on Plague [13] came, in 1952 to the conclusion that

> in pneumonic plague administration of sulfonamides or wide spectrum antibiotics might be necessary in order to prevent secondary infections or their sequels [1]

The dosages of streptomycin used by the different workers varied considerably, the more so as some merely initiated treatment with this antibiotic, and then continued it with sulfonamides while others began to use streptomycin only when the sufferers did not respond to treatment with sulfonamides and/or serum

In relatively mild cases of bubonic plague Sokhey and his collaborators [184] used an initial dose of 2/3 g followed by 1/3 g every 4 hours until the temperature had remained normal for 24 hours In severe cases, they gave 2/3 g of the drug every 4 hours until the temperature had remained normal for 2 3 days

As summarized by Girard,[68] the dosages of streptomycin administered intramuscularly for the treatment of primary pneumonic plague in Madagascar were 0 50 g every three hours for the first two days and every four hours on the third day Doses totalling 2 g per day were then given until the fifth or sixth day of treatment Girard noted that the two patients treated by Estrade had been given a total of 25 g of streptocmyin in six days but expressed the view that it offered no advantage to give more than a total of 20 g to adults He added that a child had been cured with a total dose of only 11 5 g

Girard s opinion was endorsed by the WHO Expert Committee on Plague, [13] which at its second session in 1952 recommended that

> the treatment of pneumonic plague should be carried out with streptomycin in the dosage of about 16 to 20 g administered during a period of from 6 to 7 days The same treatment should be applied to severe cases of bubonic plague with septicaemia

Other antibiotics

Dealing with the results of antibiotic treatment of mice which had been infected experimentally with either bubonic or pneumonic plague Meyer [133] stated that

> Aureomycin chloramphenicol and terramycin are good alternatives in plague therapy Each has been effective in experimental mouse infections when given systematically

or orally Oral administration gives these agents a distinct advantage over streptomycin in moderately severe cases of plague They are less bactericidal in vitro than streptomycin against the plague bacillus Thus far treatment with none of these has resulted in growth of resistant strains as readily as did streptomycin therapy

Favourable results obtained through treatment of subcutaneously plague infected mice with aureomycin and chloramphenicol were also reported by Sokhey & Habbu [180] who found these antibiotics which were given by mouth to be as effective as subcutaneously administered strepto mycin However in order to obtain identical results 42 mg of aureomycin or 336 mg of chloramphenicol had to be administered as against 10 4 mg of streptomycin

Ramchandran [160] recently recorded the results obtained with aureo mycin treatment in 12 bubonic and 3 septicaemic plague patients Three of these including two with septicaemic plague died within a few hours after the onset of treatment All the others were cured through administrations of aureomycin totalling from 2 5 g to 7 5 g given in individual doses of 250 mg every two hours The temperature of the treated became normal after 3 7 days

Some further satisfactory experiences with aureomycin in the treatment of bubonic plague were recorded by Meyer et al [138] and by Smadel et al [1 7]

A first attempt of Mercier [130] to cure a pneumonic plague patient with chloramphenicol gave no conclusive results because it was decided to continue treatment with streptomycin after two one gram doses of the former antibiotic had been administered subcutaneously

However soon afterwards favourable results were obtained when treating one bubonic and further pneumonic plague patients with chlor amphenicol alone (Girard [68] Mercier [130] Mercier & MacCrumb [131])

Mercier & MacCrumb used the following dosages for the first pneu monic patient whom they treated with chloramphenicol

1 Initially 3 intravenous injections of 0 5 g given at intervals of 3 hours every time accompanied by oral administration of 0 5 g of chlor amphenicol

2 Afterwards 3 or 4 doses of 0 5 g each given orally at intervals of 3 hours

3 Then oral administration of 0 25 g every two hours until the tempe rature dropped to 37° 37 5°C

4 Finally for a few more days further 0 25 g doses sufficiently frequent to result in the administration of a daily total of 50-60 mg of chloramphenicol per kg of body weight

The total dosage amounted thus to 20-25 g

Mercier & MacCrumb added that they afterwards treated five more pneumonic plague patients with chloramphenicol four of whom were cured The fifth whose treatment was started late succumbed These

two workers also cured two pneumonic plague patients with oxytetracycline, using somewhat lower initial doses than those given in the case of chloramphenicol but then continuing treatment in a manner similar to that in which the latter antibiotic was administered

They concluded that

> " *Terramycin [oxytetracycline] deserves to take its place side by side with streptomycin and chloromycetin amongst the antibiotics efficacious in human pneumonic plague* [m]

It is important to add that Smadel et al [177] effected a rapid cure when instituting treatment with chloramphenicol soon after the onset of fever in a 4 year old boy suffering apparently from *primary septicaemic plague*

Comparative Value of the Methods of Specific Treatment

Returning to the question of whether, in view of the supreme results obtained in the treatment of plague with antibiotics the use of plague serum might be given up, the following may be stated

Though general agreement has been reached that the results of treatment with plague serum alone are far inferior to those obtainable with antibiotics and are even inferior to those obtainabel with sulfonamides, a combination of the administration of the latter with serotherapy has been advocated by some workers in order to combat toxaemia Similarly, Girard [68] recently recommended that serum administration should be combined with streptomycin treatment because the specific serum had antitoxic properties while streptomycin, like the sulfonamides had none However in view of the almost miraculous power of this antibiotic to cure even most of the patients suffering from plague in its severest forms, it is impossible to believe that the supposed absence of antitoxic action of streptomycin is of any practical importance in the treatment of human plague Moreover other antibiotics found efficacious for plague treatment exert an antitoxic action *It follows that if sufficient suitable antibiotics are available for plague treatment there no longer exists a need to use specific serum*

In the opinion of the present writer the same holds true if large scale use has to be made of sulfonamide treatment For even though in the experience of a number of observers combined treatment with plague serum and sulfonamides gave better results than administration of the latter drugs alone there can be no doubt that far better results can be obtained by using antibiotics in addition to sulfonamides In other words the present writer for one believes that the money and effort now spent

m " La Terramycine mérite de prendre place désormais aux côtés de la Streptomycine et de la Chloromycétine parmi les antibiotiques efficaces à l'égard de la peste pulmonaire "

in producing plague serum could be used to far better advantage for the production or procurement of antibiotics suitable for plague treatment

The sulfonamides on the other hand continue to be of importance for the treatment of plague Owing to lack of funds and sometimes also of sufficient qualified medical workers to give treatment with antibiotics so far no full advantage (or in some areas no advantage at all) could be taken of this mode of therapy Indispensable though it is to remedy this deplorable situation, one should facilitate the efforts made in this direction by using as far as possible sulfonamides in combination with or in place of antibiotics In this connexion it should be kept in mind that if treatment is started early in bubonic plague satisfactory results can be obtained with sulfonamides alone, and also that, as shown by ample recent experiences patients who fail to respond to such initial treatment can almost always be saved if antibiotics are resorted to afterwards Hence unless fully sufficient amounts of antibiotics are available it seems legitimate to adopt the following scheme of plague treatment

(1) Every possible effort should be made to detect plague cases early in the disease and to start adequate treatment immediately

(2) Bubonic plague patients detected early as well as those seen later who suffer from a benign form of the disease may be treated with sulfonamides

(3) If patients treated with sulfonamides alone take a turn for the worse antibiotics must be used in addition to these drugs

(4) Treatment with antibiotics must be substituted if patients treated with sulfonamides show signs of toxic reactions due to this medication

(5) Patients suffering from primary pneumonic plague and from "septicaemic" plague and also sufferers from bubonic plague who because they have come for treatment late in the disease are in a serious condition or who show complications such as meningeal involvement must by all means from the first be treated with antibiotics

(6) Constant attention should be given to the possibility of using reduced dosages of antibiotics in combination with sulfonamide treatment or of continuing treatment of patients who have responded well to the initial administration of antibiotics with sulfonamides

Whether streptomycin will remain the antibiotic of choice for plague treatment remains to be seen The possibility that streptomycin treatment with sub optimal doses might lead to the appearance of drug resistant plague strains deserves attention in this connexion However as pointed out by Meyer and Quan [13a]

"Fortunately in the treatment of the individual patient this resistance is of minor importance since it is independent of resistance to sulfonamides Collateral therapy with sulfadiazine inhibits the streptomycin resistant strains until the immunity mechanism of the infected host is fully developed Equally the epidemiologic importance is in sign

ficant since drug resistant strains are as a rule confined to closed necrotic lesions and the infection does not become generalized

The necessity of administering streptomycin parenterally is a serious drawback particularly if qualified medical workers are few and far between Hence, while it would be rash not to make large scale use of streptomycin until more is known about the value of other antibiotics in the treatment of human plague further studies on the efficacy of the latter must be energetically pursued

General and Supporting Treatment

There is no need to enter into an elaborate discussion of the general and supporting treatment to be given to plague patients, because this should be administered according to the methods adopted in cases of serious acute diseases in general However, apart from the precautions to be taken against a spread of the infection from the sufferers which will be dealt with in chapter 10 one must give special consideration to the following points when attending plague patients

Rest in bed

It is of vital importance to keep the patients strictly confined to bed during the acute stage of the illness as well as during early convalescence Failure to abide by this rule may lead to sudden death from heart failure and, moreover, strict rest during the acute stage of illness is essential to prevent or to limit a generalization of the infection That exertions on the part of the patients are apt to lead to an aggravation of their condition is proved by observations showing that (*a*) plague patients who had to walk considerable distances to seek treatment almost invariably became seriously affected, (*b*) patients who fell ill while travelling were particularly prone to develop secondary lung involvement

The patients should not even be permitted to get up in order to void urine or stools

Delirious or comatose patients must be watched with constant care because they may try to escape or may fall out of bed and hurt themselves

Diet and fluid intake

Plague patients ought to be given a nourishing, though adequately light, diet An ample fluid intake generally necessary on account of the feverish condition of the patients is particularly important for those treated with sulfonamides The urine output of these patients should be watched preferably measured, and the urine should be examined at least once daily for the presence of albumen and/or blood

Supporting treatment

Proper supporting treatment adapted to the individual needs of the patients is essential. Whenever cardiovascular failure threatens, cardiacs such as coramine or, as several recent workers recommended, adreno-cortical preparations should be administered. The patients must be watched day and night in order not to overlook a sudden collapse.

Adequate amounts of alkalis must be given to the patients under sulfonamide treatment. Girard[6] stated in this connexion that renal complications due to the administration of these drugs might be avoided if enough alkalis were given to maintain the urine at a pH of about 7.2.

Treatment of the buboes

It is of vital importance to adhere to the following rules for the local treatment of the buboes:

(*a*) During the acute stage of the disease manipulation of the buboes must be avoided as far as possible. An incision of the affected lymph nodes at that time or the rubbing in of ointments or other medicaments is strictly contra-indicated.

In the experience of the present writer it is best to apply to the acutely inflamed buboes ichthyol or ichthyol-belladona ointment spread on gauze pads, because this medication alleviates the pain without irritating the skin.

(*b*) If pus forms, the buboes should be incised only when fluctuation has become general. Otherwise sinuses may form which may greatly retard the process of healing or even endanger the patients' lives.

(*c*) Plague workers with little experience in surgery often try to benefit the patients by frequently attending the incision wounds. Actually these heal best if interfered with as little as possible. In particular, one should change the gauze strips draining the wounds as rarely as feasible.

Abortive Treatment

Injections of moderately large amounts of specific serum have been made in the past, and medication with small doses of sulfonamides is used at present, to forestall the appearance of manifest plague in persons definitely known to have been exposed to infection, particularly in contacts with pneumonic plague patients.

Some authors use the terms "serum prophylaxis" and "chemo-prophylaxis" to designate these procedures. It seems preferable, however, to class them as methods of abortive treatment, so as to indicate that these procedures have little in common with the prophylactic administration of plague vaccines and/or small doses of plague serum to large

groups of individuals considered to be at risk of infection in areas where plague outbreaks are present or threatening

Plague serum

As can be gathered from *A treatise on pneumonic plague* [16] and subsequent publications by Wu Lien teh [17] and by Wu Lien teh & Pollitzer [19] ample use has been made in the past of the administration of smaller or larger doses of specific serum to pneumonic plague contacts Summarizing the observations made up to 1926 Wu Lien teh [16] stated that

It has been shown that prophylactic serum often prolongs the incubation period and that patients receiving such preventive treatment are specially benefited by subsequent therapeutic administration On other occasions anti plague serum when given in sufficient doses seems to prevent the manifestation of plague pneumonia

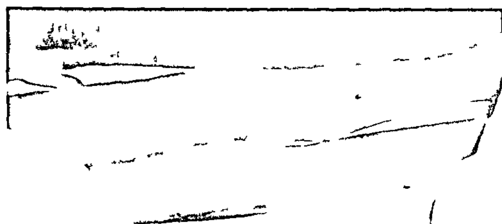
Wu Lien teh added that though, on account of experimental findings a dose of 100 ml was recommended for the abortive treatment of pneumonic plague, dosages of 40 ml to 50 ml might be sufficient if the serum could be administered early Failing that, or if there was particularly great danger of infection doses of 100 200 ml might be advisable In view of the expense involved and of the necessity to isolate the serum treated contacts for a longer period than usual, because of a possibly prolonged incubation period it would be impracticable to use abortive serotherapy during large pneumonic epidemics However the method might be practicable and advantageous for the purpose of limiting incipient outbreaks

Within recent years but little use seems to have been made of abortive serotherapy It would appear that good experiences were made with administration of 10 or 20 ml of plague immune serum to 47 contacts isolated during a pneumonic plague outbreak in Portuguese India (Figueiredo [49]) On the contrary administration of 60 ml of plague serum to contacts encountered during the 1939 pneumonic outbreak in the Chimborazo province of Ecuador seems to have given no satisfactory results since some of the persons so treated developed manifest plague (Suarez [189])

Clark & Goldberg [21] gave in addition to plague vaccine serum doses —usually of 50 ml in the case of adults and of 25 ml in the case of children— to 35 persons who had been in close contact with pneumonic plague patients Only three of the serum treated contacts developed the disease and one of these was cured through administration of further doses of 400 500 ml of plague serum It is noteworthy that (*a*) one woman who had been given 100 ml of plague serum because she had attended four pneumonic patients remained well, (*b*) three children who had slept with their mother up to and including the day she died from pneumonic plague and who then each received 50 ml of specific serum also did not develop the disease

Pinotti & al [15] as well as Sorol & al [18] reported good success when administering serum to persons who had been in contact with bubonic plague patients

FIG 32 PLAGUE CARBUNCLE IN MAN INFECTED THROUGH BITE OF WILD RODENT FLEA



Though it will be gathered that some success has been obtained with abortive serotherapy this method has become obsolete because sulfonamides besides being far more easily applicable and—in contrast to plague serum—not undergoing losses in potency during storage have also been found to be far more uniformly reliable for forestalling the development of the disease in contacts with plague patients

Sulfonamides

The possibility of using sulfonamides for the abortive treatment of plague seems to have been explored first clinically by Estrade (quoted by Girard [6]) and by Girard [6] and experimentally by Macchiavello [117] Pollitzer [154] who working in the interior of China was not aware of the above mentioned clinical experiences also obtained a most encouraging result during the second World War

Details of this early work may thus be summarized

Estrade (quoted by Girard [6]) started to give daily doses of 2 g of sulfapyridine for three consecutive days to three pneumonic plague contacts as soon as they showed fever Their temperature fell within 24 hours and no manifest illness developed in any of these three individuals Estrade felt convinced that these contacts who were under observation in an isolation camp had had incipient pneumonic plague

Girard[67] not only obtained an equally good result when treating a pneumonic plague contact, who clinically showed only some fever with sulfapyridine (a total of 11 g given during eight consecutive days) but was also able to confirm that the saliva of the woman in question collected before commencement of treatment, contained virulent plague bacilli[n]

As Macchiavello summarized in 1949[117] he had been able to establish, through experiments commenced in 1941, that treatment with sulfapyridine, sulfadiazine, and sulfamerazine, started before or within 24 hours after infection, protected 50%-75% of guinea pigs inoculated with 1,000 virulent plague bacilli—provided that doses of these drugs sufficient to maintain a blood level of 5 mg per 100 ml were administered Sulfathiazole used under the same conditions gave even better results (68% 92%) but had to be given in higher doses and was more toxic

Pollitzer[154] had full success when after the death of a pneumonic plague patient, he gave 3 g of sulfathiazole daily for three days to the two men who had closely attended the sufferer throughout his illness, one of the two even sleeping in the sick chamber

Further observations made with regard to abortive plague treatment with sulfonamides may be summarized thus

Pneumonic plague Meyer[13] established experimentally that guinea pigs instilled intranasally with *P pestis* failed to contract pneumonic plague when given sulfadiazine or sulfamerazine at the time of infection

Though occasional failures have been noted (Le Gall,[10] Favarel et al [47] Jospin et al [9]), actual experience with sulfonamide treatment of pneumonic plague contacts in Madagascar was in general, most satisfactory Favarel et al [47] stated in this connexion that

> is used systematically in Madagascar to treat all known pneumonic plague contacts without awaiting the appearance of any pathological manifestation Either MB693 (Dagenan) [sulfapyridine] or RP 2090 (Thiazomide) [amino phenyl sulfamidothiazol] are used in a daily dose per os of 4 g for adults 3 2 g for children according to their age Dagenan and thiazomide gave up to now practically 100/ success in the prevention of pneumonic plague in contacts [o]

Favarel et al [47] added that treatment of the contacts was successful even in cases where these individuals had to be left in their houses instead of being taken to isolation camps

Similarly Girard[67] stated in 1951 that

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[n] An identical result obtained by Robic (quoted by Favarel et al [4]) in the case of a boy found to harbo r *P pestis* in his saliva before abortive treatment with sulfonamides was commenced serves as corollary to Girard s observation

[o] Elle est appliquée systemat quement a Madagascar depu s plus eurs a inées chez tous les contacts connus de pesteux pulmonaire et sans attendre chez eux la mo ndre manifestation patholog que On utilise soit le 693 MB (Dag nan) soit le 2090 RP (Thiazomide) à la dose quot dienne *p r os* de 4 g pour les adultes et 3 à 2 g pour les enfants suivant l age Le Dagenan et la Th azom de ont pratiquement donné jusqu ici à Madagascar 100/ de succes dans la prévention de la peste pulmona e chez les contacts

in contrast to serum prophylaxis sulfonamides (Dagenan Thiazomide) are efficacious in a dose of 3 g per day for 6 days It is exceptional now to see the development of pneumonia in contacts who have been spotted isolated and treated early This is a considerable progress confirmed by 10 years experience in Tananarive [p]

Pollitzer[151] was able to suppress an incipient pneumonic plague outbreak in a rural area near Tsinkiang China by giving sulfadiazine, in doses varying according to degree of exposure and age to 26 contacts None of these individuals, who were left in their houses, developed the disease

Results recorded by other observers were as follows

Locality	*Reference*	*Date*	*Number treated*	*Drug and dosage used*	*Instance of infection*
Oran (North Africa)	Roux & Mercier[167] Gordon & Knies[73]	1946 1947	85	Sulfathiazole (3 g daily apparently for 7 days)	One (patient recovered)
Iranian Kurdistan	Baltazard[2]	1948	?	4 g sulfadiazine or sulfathiazole per day Treatment was continued until 2 days after the death of the last victim in the house concerned	None
Nanking (China)	Huang et al[8]	1948	64	Sulfadiazine—6 g daily for 1 week to close contacts 3 g daily for 1 week to those under lesser risk	None
Mukden (China)	Tieh et al[19]	1948	4 (all found to harbour *P pestis* in their throat)	Sulfadiazine (4 12 g for 4 6 days)	None
Foochow (China)	Feng[45]	1949	33	Sulfadiazine (4 g on first day of treatment then 3 g for 6 days for adults half of these doses for children)	None
Copshi (Ecuador)	Sáenz Vera[168]	1949	40	Adequate doses of sulfathiazole " (apparently 3 g daily)	None

Note A failure of abortive treatment with sulfapyridine was reported by Plum[153] in Nairobi Kenya

[p] Contrairement à l'échec de la séroprophylaxie les sulfamides (Dagénan, thiazomide) sont efficaces à la dose de 3 g par jour pendant 6 jours Il est maintenant exceptionnel de voir éclater un cas de peste pulmonaire chez des contacts repérés isolés et traités précocement C'est un progrès considérable que confirme une expérience de 10 années à Tananarive

As will be noted, the dosages used by the different workers, and the number of days for which they administered the sulfonamides chosen by them varied considerably The standard *recommendation made in this respect* by the WHO Expert Committee on Plague in 1952 [213] was the administration *of daily doses of 3 g of a suitable sulfonamide* over a period of 6 days

Bubonic plague As recorded by some workers such as Gordon & Knies,[73] Lewis et al [11?] Macchiavello,[117] and Sáenz Vera,[168] administration of sulfonamides to contacts with bubonic plague patients was effective Erzin & Payzin [38] *had good results when giving plague serum* as *well* as sulfonamides to such contacts

However, as pointed out by Pollitzer [154] it would not be advisable to make large scale use of abortive sulfonamide treatment when dealing with bubonic outbreaks because

during these the danger of human infection is much less limited as to space and time than is the case in lung pest where familial explosions are the rule *In rat-caused* and flea borne plague on the contrary where as a rule merely off-chances for human infection exist the cases are usually spaced out and in the great majority of instances we find but one victim per house To administer chemo prophylaxis (scilicet abortive treatment) to the contacts of patients suffering from bubonic plague without lung complications seems therefore as a rule not *indicated* To use *the method on a settlement* or precinct wide scale would be quite expensive and seeing that the protection thus conferred is of a rather ephemeral nature, *the value of the procedure would be questionable*

Certainly, however, it is imperative to administer abortive treatment with sulfonamides to contacts of bubonic patients with lung involvement One should also not hesitate to use this method for the purpose of warding off bubonic plague attacks if special conditions, such as the occurrence of laboratory or postmortem accidents or the occurrence of plague on a ship or *in a port hitherto free of plague warrant this It must be realized* however, that, in general, other methods are of prime importance for the control of bubonic plague

To administer antibiotics, instead of sulfonamides, for abortive plague treatment would be inadvisable, if for no other reason than that the danger of producing drug resistant strains would be far greater when limited amounts of the antibiotics were used for this purpose than when large doses were given for the treatment of manifest plague attacks One should realize, therefore, that while the antibiotics are of supreme importance for *plague treatment in general*, sulfonamides are the drugs of choice for the special purpose of abortive treatment

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Chapter 9

EPIDEMIOLOGY

INTRODUCTION

In dealing with the epidemiology of plague as will be gathered from chapters 4 and 8 two fundamentally different forms of the disease should be considered

(1) bubonic plague produced as a rule by the bite of plague infected insect vectors mainly rodent fleas

(2) primary pneumonic plague due to a spread of the infection from man to man

Even though free living infected rodent fleas may be temporarily important in the causation of bubonic plague in man the continued existence of this form of the disease depends in the long run upon the persistence of the infection in the rodents The name of zootic plague recently recommended [88] for this form of the disease is therefore most appropriate

Though initially dependent upon a spread of the infection from bubonic plague patients with secondary lung involvement to members of their families or other contacts primary pneumonic plague is apt to spread regardless of whether or not infected rodents and/or infective fleas continue to be present in the localities in question In fact purely pneumonic plague epidemics due to the arrival of patients with secondary or primary lung involvement have quite often been observed in localities where the rat populations were originally free from plague and where they remained entirely so even when the disease became rampant in man

No doubt therefore primary pneumonic plague well deserves the recently proposed [88] designation of demic plague—the more so because just as in the so called bubonic outbreaks cases without apparent buboes are met with so in pneumonic epidemics pulmonary cases without manifest lung involvement occur and rare bubonic cases may be produced through

contact infection or, exceptionally, through the bite of infected human ecto parasites

BUBONIC (ZOOTIC) PLAGUE

Cause of the Outbreaks

As noted in chapter 6 the Plague Research Commission was able through a series of well planned experiments to disprove that infection through the air through direct contact, or through contaminated inanimate objects was of any importance in the transmission of plague from rat to rat, and to establish on the other hand, that plague was principally an insect borne —and particularly a flea borne—infection

At the same time, the Commission adduced convincing proof that what had been established in regard to the spread of the infection among the rats, also held true for the conveyance of bubonic infection to man As summarized by Lamb[40, 41] the Commission found, in this respect in particular, that

(1) *A direct spread of the infection from bubonic patients was most* unlikely, because (*a*) their excreta as well as those of the rats were found to be non infectious when tested in the laboratory under conditions analogous to those in nature and (*b*) the pus of healing buboes contained few, if any virulent *Pasteurella pestis*

(2) There was no convincing evidence to show that the human flea played an important role in the conveyance of the infection to man

(3) Those attending bubonic plague patients remained singularly free from infection, the plague hospitals being in fact the safest places during the outbreaks

(4) The contacts of patients who developed bubonic plague after arrival *in a hitherto unaffected locality invariably remained well*

(5) The great majority of the patients whose history was accurately known had had no contact with previous cases before falling ill

(6) If bubonic plague appeared in a settlement, in the great majority of instances not more than one case occurred per house

(7) If multiple cases occurred in any house, they often appeared simultaneously as if infected from a common source

(8) *If successive cases appeared in a house invariably* there was evidence of a higher rat mortality than that found in houses yielding single plague cases

The Plague Research Commission also established that close relationships existed between *Rattus rattus* epizootics and bubonic epidemics in time, in place, and in quantity

As summarized by Wu Lien teh [98] in Bombay City the average time interval between the epizootics and the appearance of the epidemics was 10-14 days and in a Punjab village about a week—the difference being due to the fact that the Bombay figures were based on plague deaths and those for the village on attacks

The period of 10 14 days observed in Bombay comprised (*a*) three days which according to laboratory observations had to elapse before the fleas coming from the dead rats were willing to attack man (*b*) the average incubation period of human plague (three days) and (*c*) the average length of illness (5½ days)

The observations of the Commission also left no doubt that the places of infection in Bombay were the same in the case of *R rattus* and in that of man The same held true for the Punjab villages but there account had to be taken of the close aggregation of the houses and the intercommuni cation of rat burrows between neighbouring habitations

However the Commission laid stress upon the fact that evidently because many plague rats died in their burrows infected fleas could be trapped in houses where no rat falls had been noted

A close relation in quantity was found to exist between the *rattus* epizootics and bubonic epidemics If no rats with acute plague were found during the off seasons human cases were also absent During the seasons the epidemic curve closely followed that of the *rattus* epizootics An intimate relationship also existed between the number of plague rats found in a house and the appearance of human plague Thus in one of the villages studied human cases occurred in only 3.4% of the houses in which single plague rats had been found whereas they occurred in 28% of the houses in which more than one plague rat had been found

Generally speaking the conclusions reached by the Plague Research Commission have remained unchallenged up to the present In particular it has been confirmed through ample observations in various plague areas that in contrast to the demic form of plague the plague cases appearing during zootic outbreaks in individual houses usually remain single

As has been discussed in chapter 7 some subsequent observers have maintained that in addition to or even instead of rodent fleas human ectoparasites particularly *Pulex irritans* are of importance in the trans mission of bubonic plague While admitting that in places where these ectoparasites abounded they might play a role in this respect it was stressed that in most plague areas particularly those in China India and Madagascar the part taken by human parasites in the conveyance of the infection was negligible the transmission of plague to man depend ing upon the rat fleas It was admitted however that the importance of wild rodent fleas in the conveyance of the disease to man was not as universal human infections in the sylvatic plague foci being partly due to direct contact with diseased rodents

Attention was also drawn to observations *showing that cases of tonsillar* plague were found to be frequent among the Indians of Ecuador, who were in the habit of killing fleas and lice caught by them with their teeth

A few observers such as Durand & Conseil[18] and Nikanoroff[56] conceived the idea that human convalescent or healthy carriers might be of importance in the perpetuation and spread of bubonic plague, the former two authors postulating that individuals harbouring *P pestis* might develop plague bacteraemia if they became the prey of intercurrent diseases such as influenza or measles However, no definite evidence has ever been brought forward to support these assumptions and there seems no reason, therefore, to revise the opinion expressed by Wu Lien teh[98] that 'like rodents with chronic plague, human carriers represent so to speak, a sidetrack of the infection which ends blindly '

While it is generally admitted that even though plague infected rats are found in individual houses or compounds human cases do not invariably follow in them, the opinion, held by most workers, that rat plague may exist in a community without leading to the appearance of the disease in man has not been universally shared Petrie,[60] in particular seems to have been sceptical in this respect Discussing the discovery of ' clandestine " foci of rat plague in Java by Swellengrebel & Hoesen[85] he pointed out that the absence of human cases in these localities might have been merely apparent, because the methods available for case finding were far less exact than the pooling tests used to detect the presence of *P pestis* in the rats and their fleas

There can be no doubt, however, that—as proved by recent experiences—rat epizootics may run their course without leading to human plague The paucity or even absence, of human cases in foci of wild rodent plague also deserves great attention in this connexion

Trend of Epidemics

In correlation with the slow evolution of the causative epizootics and the consequent restriction of the number of flea vectors initially available for human infection, the onset of bubonic epidemics is, as a rule rather gradual Hand in hand with an increasing rodent mortality and a corresponding increase of the infection quantum ' represented by infective rodent fleas, the outbreaks spread and reach the stage of their full development This period of maximal morbidity and mortality is followed by a third stage of gradual decline of the epidemics (Dieudonne & Otto[16]) The evolution of bubonic outbreaks is thus markedly different from that of pneumonic epidemics which develop far more rapidly However in contrast to the latter which though apt to become rampant are usually of an episodic nature, bubonic manifestations, because they serve so to speak

merely as an index of a persisting rodent infection tend to recur—often over prolonged periods—whenever conditions for a transition to man become suitable From what has been discussed in chapters 6 and 7 it will be gathered that this periodic or one should say more accurately "seasonal incidence of bubonic plague outbreaks is governed by changes in the climatic conditions which mainly by altering the infectivity of the flea vectors exert a powerful influence upon the trend of the epizootics and consequently a most marked though indirect influence upon that of the epidemics

The data furnished in chapter 1 regarding the seasonal incidence of plague in the recently or currently affected areas may thus be supplemented and summarized

Area	*Plague seasons*
China	*North China* Outbreaks started in late summer or autumn some times extending into the cold season (Wu Lien teh [98]) *Fukien Province* Commencement in spring (usually between April and June) Epidemics lasting until early autumn sporadic cases occurring as late as October and November (Park [59]) *Kwang tung Province* February June (Park [59]) *Hainan Island* February April (Landauer [4])
Burma	The usual plague season falls in the period November to April with a peak in February in Upper Burma and in March in Lower Burma *where there may be a secondary rise in July (Park [59] Wilcocks [95])*
Viet Nam (South)	The first three quarters of the year with a peak in April May (Park [59] Herivaux & Toumanoff [3])
Thailand	According to Bangxang [3] case incidence begins to increase in September reaching its highest point in February and March Low level from May to September particularly during the wet months of June and July
Java	Seasonal incidence not pronounced because despite the presence of a dry and a wet monsoon the temperature remains practically uniform throughout the year Still plague mortality begins to increase in the third quarter of the year reaching its maximum in December or January (middle of wet season) commencing to decrease in February and continuing to decrease in May and June (dry season) (Park [59])
India	Dealing merely with the areas which were chiefly affected within recent times Park [58] stated that Although plague is at a minimum during the hot and dry summer months which in general all over India are from March to May the incidence during the colder months varies in the different provinces owing to the varying rainy season which in turn controls both the lowering of temperature and the rise in humidity The South west Monsoon breaks on the west coast of India early in June and provides suitable climatic conditions to permit in Bombay Presidency a peak incidence in October Thereafter the infection maintains a fairly high level till the end of the year only to decline in the heat of March April of

Area	Plague seasons
	the following year Madras Presidency is subject to both the South-west and the North-east Monsoons and its seasonal incidence is thus more complicated but on the whole it provides a peak incidence much later than Bombay in January in common with the neighbouring States of Hyderabad and Mysore The North Indian Provinces of United Provinces and Bihar are seen to have their peak incidence in March and in common with the rest of India the incidence declines sharply with the dry heat of the second quarter"
Egypt	As stated by Kamal[37] (see also Wakil quoted by Pollitzer[63]) the months of maximal plague prevalence are in northern Egypt (which has a Mediterranean climate) June and July in middle Egypt May in upper Egypt (which has a hot and dry climate tempered by northern winds) March and April
Tunisia	Outbreak at Ferryville observed by Magrou[48] lasted from Aug 1944 until March 1945 Percentage incidence of *Xenopsylla cheo* was according to Meunier[51] highest in summer
Algeria	Incidence highest in August (mean temperature 80°F (27°C) plague then declining with the increased rainfall and falling temperatures from September onwards (Davis[13])
Morocco	According to Davis[13] it could be noted that plague is most prevalent at Casablanca in May and at Marrakesh in June and that in both areas there are two peaks during the year—namely in May June and October at Casablanca and in June July and November at Marrakesh The hot weather of midsummer seems to have a depressing effect on plague incidence in both areas
Senegal	Davis[13] stated that The most prominent feature of the seasonal epidemiology of plague in Senegal is the sharp rise after the dry period January February (when the dry harmattan winds blow) to a peak in June before the onset of the rainy season during July September There is a noticeable recession in the two hottest months of the year—namely September and October The rise in temperature and relative humidity before the rains seems to favour human plague transmission which is checked by high temperatures at the end of the rainy season (October)
East Africa (Belgian Congo Uganda and Kenya)	Dealing with the three following East African plague regions Davis[13] stated that the equatorial climate at relatively high elevations with rather slight variations in mean temperature throughout the year appears to favour a uniform distribution of human plague without marked seasonal peaks *Belgian Congo* Case incidence lower between December and April higher between May and November with a slight recession in August and September the periods of lower incidence correspond to the drier times of the year and the first months of the long rainy season which follow the dry season of December and January (Davis[13]) *Uganda* Seasonal plague incidence similar to that in the Belgian Congo According to Hopkins[33] all endemic foci are situated in areas with a rainfall above 45 inches (1 140 mm) *Kenya* Though cases were recorded in all months Roberts[70] found during an outbreak at Keruguya a rise in the case incidence at the time of the harvest the incidence becoming highest between the two rainy seasons

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Area	Plague season
East Africa (Tanganyika)	As emphasized by Davis[13], in contrast to the fairly uniform seasonal distribution of plague incidence in the above mentioned East African regions, there is a well marked plague season in Tanganyika which, lasting from February to April, corresponds to the single rainy season. The tropical climate prevailing during the rest of the year obviously prevents an incidence of human plague.
Madagascar	According to Robic[2] the climatic conditions prevailing in the hot and damp coastal areas are unsuitable for the persistence of plague, while the relatively cool and moist weather on the high plateau creates a most favourable environment for its continued existence. Though cases occurred there throughout the year, plague regularly assumed epidemic features during the period of October to March when, as recently summarized by Girard[30], free living *X. cheopis* abounded in the houses where plague cases had occurred and were frequently infective. During the cool and rainless winter months when the fleas appeared to be inactive, the incidence of bubonic plague was lowest. However, as stated by Le Gall[43] and by Girard[30], pneumonic plague cases were comparatively more frequent in winter.
South Africa	As stated by Davis[12], in the Union of South Africa plague epizootics may be in progress at all times of the year. Likewise the secondary infections of commensal rodents, which are directly responsible for human manifestations of the disease, may take place at any time. Nevertheless, human attacks were most frequent in summer. The seasonal incidence of human plague in Basutoland, in South West Africa, in the Bechuanaland Protectorate and in Northern Rhodesia corresponded to that in the Union of South Africa (Davis[13]).
USA (western States)	According to Meyer[5], epizootics among the ground squirrels which led to the appearance of sporadic plague cases in man began early in spring, rose in intensity during the summer months and slowly declined during autumn to disappear entirely during the winter in regions where the animals hibernated. However, in some localities young ground squirrels, which were apt neither to aestivate nor to hibernate, could be found plague infected in December and January.
Hawaii	As summarized by Mohr[53], the *cheopis* infestation of the Honolulu Norway rats was lowest in October and highest in January. June also appeared to be a high month, but May and July showed low infestation rates. Mohr added that, since temperature and humidity at Honolulu were high but not too high for *X. cheopis*, infestation of the rats with this flea was marked.
Venezuela	As noted in chapter 1, the 1943 plague outbreak in Aragua State appeared in July, i.e., one month after an increased incidence of *X. brasiliensis* had been noted.
Brazil	From chapter 1 it may be gathered that bubonic plague was most frequent at São Paulo in the south of the country in summer with a peak in January. In the north-east of Brazil, on the contrary, the incidence of the disease was comparatively highest from July to October, while the onset of the epidemic season in Ceará, which is still farther north, was in May and June. Dealing with the epidemiology of plague in Brazil in general, Barreto & Castro[6] noted an increase of the case incidence in spring

Area	Plague seasons
	or autumn when following periods of heaviest rainfall the temperature ranged from 19°C to 26°C (66°-79°F) and the relative humidity from 66% 83%
Argentina	While as maintained by Villafane Lastra et al [91] the plague incidence was formerly highest in summer the recent rural outbreaks occurred mainly in winter (Sussini, [81] Barrera [4])
Bolivia	According to Siles [78] both summer and winter outbreaks of plague were observed
Peru	Generally speaking the annual plague epidemics in Peru tended to reach their peak during the summer months but in areas where the winter months were warm the plague seasons fell into an earlier period than in localities with a colder climate (Eskey [19])
Ecuador	Plague was generally most rampant in December of low incidence in June However the outbreaks in Loja Province occurred mainly during the dry season from May to December (Moll & O Leary [54])

At first glance, the data assembled in the above tabulation appear to be rather contradictory, because it will be noted that in some of the plague areas the case incidence was highest during the warm seasons and in others during the cool seasons, and that the prevalence of rain sometimes favoured and sometimes cut short the spread of the infection to man However, whenever it is possible to correlate the statements made in regard to the seasonal incidence of bubonic plague with adequate meteorological data, it will be found that invariably the epidemics occurred during seasons in which the temperature was moderately high and the saturation deficiency of the air low Though some exceptions appear to exist as a rule bubonic plague epidemics occurred at times when the prevailing climatic conditions were within the limits found suitable by Brooks [7] for the occurrence of such outbreaks in India

Spread and Persistence of Outbreaks

The consequences of plague invasion of the various recently or currently affected countries which as can be gathered from chapter 1, was due mainly to importations by the sea route varied according to local conditions Whenever the climate prevailing at the time of the invasion or throughout the year was unsuitable the infection failed to entrench itself It is noteworthy, however that even though owing to such unfavourable conditions, plague was unable to gain a permanent foothold in the coastal regions of Madagascar it still managed to penetrate into the interior of the island where almost ideal conditions for its persistence existed

The prevalence or sole presence, of comparatively inefficient vector fleas was also apt to impede the spread of plague particularly its transition to man Thus as pointed out by Eskey [0] in Seattle Wash, USA where *Nosopsyllus fasciatus* was the vector plague smouldered among the rats for

ten years and yet only three human cases were recorded throughout this period

However instances such as those referred to above were exceptions rather than the rule Usually the invasion of a sea port by the maritime route (or as shown in the case of Hong Kong and Canton even by the land route) was followed not only by an often prolonged though gradually decreasing local occurrence of plague but also almost invariably by a spread of the infection to the hinterland Practically without exception this led first to the involvement of inland towns connected with the ports by major traffic routes—particularly railways and rivers—and secondly to a further spread of the infection to rural areas which because it usually depended upon a transport of infected fleas by primitive means of communication was apt to take place in a rather haphazard manner For reasons which will be discussed later plague while tending gradually to disappear, or to become minimal in the urban centres of the hinterland as well as in the coastal towns showed a most marked tendency to remain endemic in rural areas Quite often the infection sallying forth from these strongholds produced epidemics in adjacent areas which no longer permanently suffered from the disease

Although it is legitimate to consider the above described mode of plague propagation which has been observed regularly in widely distant countries as typical many local variations of the process have been found to exist, owing principally to differences in the rodent species which served as reservoir of the infection While in some of the countries commensal rodents continued to harbour the infection when rural areas became involved in others the infection spread to wild rodent species Foci of true sylvatic plague could thus be formed dangerous to man only when he penetrated into the remote haunts of the species concerned In other cases however the wild rodents involved lived so near to man that their fleas could cause human infections within the settlements or could at least infect commensal rodents which in their turn brought the disease into the houses

Under these circumstances it is not surprising to find that different authors proposed different schemes for classifying the various stages of plague Thus Devignat [14] distinguished three levels (plans ") on which plague could develop—the domestic murine (murin domestique ") the " sylvatic murine (murin selvatique) and the human " ("humain") —which could be independent of one another or could be brought into relation by different modes of transmission

In a valuable study Macchiavello [47] distinguished four main stages in the propagation of plague in South America namely (*a*) invasion of the ports (*b*) spread by railways and other means of communication to the towns of the hinterland (*c*) subsequent involvement of adjacent rural areas and (*d*) transition of the infection to wild rodents

Important though the latter classification is for historical surveys, it suffices for the purposes of the present disquisition to give separate consideration only to (a) urban manifestations (b) rural manifestations, including under this heading besides rat caused outbreaks, those of a 'peridomestic' origin, due to an interchange of the infection between commensal and wild rodents or to proximity of the latter to human habitations, and (c) wild rodent plague in the strict sense of the term These three main categories will now be dealt with seriatim

Urban manifestations

As stated before, for various reasons, particularly owing to untoward climatic conditions or to the lack of efficient flea vectors, plague may fail to gain a foothold, or at least a permanent foothold in the urban centres into which the infection has been introduced However as shown by many examples, given a suitable climate a sufficiently large and susceptible rodent population, and the presence of a sufficient number of capable vectors plague often became firmly entrenched among the rats of the affected cities or major towns, and persisted in the rodent populations for at least a number of years, not rarely for many years If, as was often the case, the prevailing climatic conditions did not favour perennial epizootics, plague assumed epizootic proportions whenever suitable seasonal changes of the weather took place The high mortality of the rats during these seasons led to the liberation of infective rat fleas and consequently, to the appearance of human plague manifestations, which at least during the period immediately following the introduction of the infection practically always assumed epidemic proportions

During the off seasons, when, as a rule the surviving rats were capable of attracting the fleas which had left the few animals that succumbed to plague human cases, if occurring at all were few and far between

Several reasons have been adduced to explain the gradual diminution and ultimate disappearance of the infection, which are characteristic for urban zootic plague Thus some authors, such as, recently, Macchiavello,[47] stressed that a decimation of the rats through severe epizootics might greatly diminish or even abolish the chances of infection among the scattered survivors However, the importance of this factor is limited because urban plague often spreads in an irregular manner so that, even in severely affected precincts, groups of rats may remain unscathed Moreover, unless the reduction of the rat population becomes extreme an increased fertility of the survivors is apt to lead soon to a re establishment of the former population level or even to an increase in population As maintained in chapter 6, a vicious circle may exist in this respect, a decrease of the rat population through an epizootic leading to more frequent births during the following off season which in their turn help to promote the next epizootic

One must fear that the procedures employed hitherto for rat destruction play an ambiguous role in the control of urban plague since they also often act as a stimulus for a high compensatory fertility among the survivors That generally speaking one should not think too highly of the routine methods formerly used for plague control, is exemplified by the observations of Utley[90] in Hong Kong He concluded that the decrease of plague in that port and its final disappearance in 1923 could not be ascribed to the measures taken because simultaneously the incidence of the disease decreased in the adjacent parts of south China where no or at least no intensive control work had been done

Even at best the influence of the factors considered above is of limited importance compared to that exerted by the gradual extinction of plague susceptible rats which taking place in the course of the successive epizootics leads to an increasing prevalence of plague resistant rat strains and finally to the exclusive presence of rats refractory to the infection The evidence adduced in this respect in chapter 6 leaves no room for doubt that during the present pandemic as well as in historical times this gradual replacement of susceptible rats by resistant rats exerted a profound influence upon the trend of the urban zootic plague outbreaks and finally led to their disappearance

As has been stated in chapter 6 not much information is available as to how long this herd resistance was apt to continue in the absence of autochthonous plague It stands to reason that in such cases a gradual multiplication of a few susceptible rats which had been spared during the past epizootics might take place and that likewise susceptible imported rats might have a chance to multiply As was shown in chapter 6 such an evolution actually took place in Bombay As also observed there a change in the situation may likewise be effected by a multiplication of rodents other than the common rats which because formerly less numerous have suffered little in the past rat epizootics and therefore have not lost their herd susceptibility to plague

That under such circumstances a reimportation of plague may create a dangerous situation for man has been proved by recent experiences in Bombay which may be recorded thus

Year	Plague infected rodents found: R. rattus	Gunomys kok	Plague cases [illegible]
1948	2	42	14
1949	4	2	2
1950	0	0	2
1951	0	0	0
1952	0	0	1

It will be noted that owing no doubt to a reimportation of the infection from Bombay State an enzootic was present among *Bandicota bengalensis kok* (*Gunomys kok*) and to an apparently lesser extent also among *R. rattus* in 1948 and 1949 and was obviously responsible for a number of plague

cases among residents of Bombay who had never left the city Though no infected rodents were found afterwards, the epizootic probably continued because further plague cases among residents were recorded in 1950 and 1952 respectively It should be noted in this connexion that so far no pooling tests have been used for rat examination in Bombay

In assessing the importance of these observations one must admit that (*a*) an unusually favourable situation for the reappearance of plague has been created in Bombay through the recent multiplication of the fully susceptible *B bengalensis kok*, and (*b*) as proved by the almost all yearly occurrence of imported human cases, chances for reimportation of plague into the city were considerable

Nevertheless, though they are probably exceptional, these findings show that zootic plague may become re established in an urban community comparatively soon after it has become extinct

The extent to which plague affected urban communities serve as distributing centres of the infection may vary considerably Apart from the degree to which the cities and towns are affected, which often shows seasonal variations and is apt to decrease in due course, the role which these centres play in the distribution of plague depends, in the first place on their commercial importance and on the means of communication they possess If railways or steamers are available for traffic, a spread of plague from seriously affected urban centres particularly from those of great commercial importance, is practically inevitable and is apt to lead to an importation of the infection into even quite distant towns which then become secondary plague distributing centres Vice versa the extent to which these secondary centres spread the infection decreases pari passu with their lessened importance for commerce and traffic If they possess only primitive means of communication, they may distribute the infection merely in their immediate environs Naturally, such a spread to surrounding rural districts is bound to take place also in the case of important plague infected centres in addition to a long distance spread of the infection by modern means of communication (peste urbano rural of Macchiavello [47])

Rural manifestations

In contrast to purely " sylvatic plague the epidemiology of which will be dealt with separately, the rural manifestations of zootic plague—due to a presence of the infection in commensal and also in peridomestic wild rodents—have no independent standing in so far as practically always, their initial appearance and sometimes even their recurrence is due to an importation of the infection from some plague affected urban centre Greenwood [31] studying the outbreaks in the Punjab maintained in this connexion that such an importation of the infection was most likely to occur in the case of villages which were large and situated near major lines of communication The proportion of small villages which remained

free from infection when plague became epidemic in an area was therefore much higher than that of larger villages or towns

The problem of the persistence of plague in rural areas has been studied by numerous workers Confirming and supplementing observations made in this respect by the Plague Research Commission and other early investigators Kunhardt [39] in a paper read in 1912 at the Second All India Sanitary Conference held at Simla India drew a distinction between incomplete and complete rural plague outbreaks If the infection was introduced late in the season into a large settlement an incomplete outbreak was apt to result and plague was likely to be carried over into the next season On the contrary complete outbreaks as they occurred in small villages or in large communities which had been affected early in the plague season were not carried over apparently because the rat population had been decimated to such a degree that a further spread of the infection among the scattered survivors had become impossible Kunhardt had actually found that during the period 1899 1904 only 17 villages in the Poona district (Bombay State) had carried over the infection during the off seasons none of them more than once He disbelieved therefore that plague was endemic in the rural parts of the district

Browning Smith [8] in a paper read at the same conference was not in agreement with Kunhardt stating that in the Punjab at least the factors determining the local appearance of plague seemed to be very complex He admitted however that plague rarely appeared in autumn in a place which had had a complete epidemic early in the year Turkhud [89] who also made a report during the 1912 conference concluded from careful observations made in the Satara district (Bombay State) that during each off season plague was carried over only in one village whence the infection spread to the other villages which became involved in the same year A different village was found to be the fountainhead of the infection in each subsequent year

Acting upon the recommendation made in 1910 by the Punjab Plague Committee that in plague control work attention ought to be concentrated on places in which incomplete outbreaks had occurred Kunhardt & Chitre [39] worked out a scheme for predicting the carrying over of epidemics based upon (1) the size of the communities in question and (2) the month when the first autochthonous human plague case had occurred As summarized by Wu Lien teh [98] the two workers found that a carry over of plague was possible under the following conditions

Population of more than	*Date of first autochthonous plague case*
25 000	November
10 000	December
4 000	January
1 600	February
800	March

Strickland,[83] while agreeing in principle with Kunhardt & Chitre, pointed out with much reason that the figures arrived at by these two workers were not generally valid but that, on the contrary, a separate formula had to be worked out for each plague district Thus in his experience in the Belgaum and Dharwar districts (Bombay State), a carry over took place in settlements which were much smaller in size and had been infected earlier than had been found by Kunhardt in the near by Poona area Strickland [83] also suggested that the transportation of grain after the harvests because it facilitated the transportation of rats and fleas might lead to an increased number of village infections

According to the observations of George & Webster [34] the villages of the Cumbum valley in southern India also did not suffer from plague equally from year to year " It was, no doubt, due to this inconstancy of the plague manifestations that, as established by these two workers, the rats of the Cumbum valley were almost invariably susceptible to cutaneous or subcutaneous inoculations with *P pestis* (97 5% positive results in 320 rats tested)

Observations similar to those recorded above were also made by workers in China Landauer,[4] discussing the plague situation in Hainan, came to the conclusion that, in that island

> the infection of the villages represents a distinct phase in the epidemiology of plague The towns are infected first but the disease has a tendency to become extinct after a series of more or less violent eruptions The second stage (which does not follow necessarily) consists of an invasion of the country where the disease assumes a different character The small number of rats implicated leads to an uninterrupted chain of sporadic cases or of small epidemics comprising at most 10 persons per locality Once the disease has reached this stage it rarely shows a tendency to disappear spontaneously from the regions thus affected and very often towns which had been free from plague for a number of years are reinfected from the villages " [a]

Similar conclusions were reached by Yang et al [101] who worked in the Fukien Province They maintained that

> " scattered cases typical for the offseason are never found in towns They sometimes occur in the outskirts of towns especially among the hut population but are most frequent in isolated farms or small hamlets Due to their isolation number and small size such farms rarely become infected twice Their rat populations have had no chance to become immune and may be likened to small piles of firewood scattered over the whole region Sparks in the form of infected fleas or rodents set them on fire and when the large fire in the town has burnt out long ago for lack of combustible matter there is always a new pile of fuel available in the country side

[a] l infection villageo se représente une phase distincte dans l épidemiologie de la peste Les villes sont infectées les premières mais la maladie a tendance à s éteindre après une série d éruptions plus ou moins violentes La seconde étape (qui ne doit pas nécessairement faire suite à la première) consiste dans l invasion de la campagne ou la maladie prend un caractere différent Le petit nombre de rats impliqué conduit à une chaîne ininterrompue de cas sporadiques ou de petites épidémies comprenant au plus 10 personnes par endroit Arrivé à ce stade il est extrêmement rare de voir la maladie s éteindre spontanément dans les régions ainsi infectées et tres souvent des villes qui ont été exemptes de peste pendant un certain nombre d années sont réinfectées par les villages "

The excellent descriptions of the trend of rural plague outbreaks given by Landauer[4] and by Yang et al[101] are all the more important in view of the opinion held by some observers that the appearance of the rural manifestations is invariably the result of an importation of the infection from a plague affected urban centre Thus Sharif & Narasimham[7] recently studying the ecology of plague in two districts of Bombay State maintained that the idea that plague is more a rural problem is fallacious In their opinion the big grain centres received the infection from some infected village through fleas imported with grain and caused a dissemination of plague mainly through grain to other villages Usually, the grain centres themselves did not become seriously involved in this progress of the infection because owing to past epizootics a large proportion of their rats was plague resistant

It is undeniable that at a late stage of their infection plague affected towns may as it were serve as relay stations for the spread of the disease from one rural area to another without markedly suffering themselves However as proved by the above quoted observations in China rural plague may persist and spread after the infection has become extinct in the adjacent towns and may be responsible in due course for a reinfection of the latter

Pollitzer[6] who during the second World War had fairly ample opportunities of observing the evolution of plague in hitherto unaffected districts of south China ab initio found that if a new county became invaded as a rule rat plague followed in due course by human infection first became manifest in the county (hsien) capital or some other centre like a market town Occasionally however the disease first appeared in some village situated close to a previously infected district If so the hsien capitals or other important towns almost invariably became involved in their turn and then served as distributing centres As in other countries plague in the towns gradually decreased and then disappeared but occasionally the infection was reintroduced from adjacent rural foci

Plague was as a rule shortlived in the numerous villages attacked Appearance of the infection early in the season led to a " complete out break with numerous rat falls and human victims the marked reduction of the susceptible rat population often resulting in a total disappearance of the disease If importation took place late in the season limited epizootics with little or even no human plague developed Rat falls became few and far between after the end of the season but almost inevitably such " incomplete outbreaks led to a carry over of the infection and thus to conspicuous outbreaks in the following season

The local reappearance of plague in more than one season was rare but though not persisting for long the infection was often carried from the affected to hitherto unaffected villages usually through the transport of infected fleas in rice cargoes

The conclusion reached by Pollitzer was that, while in the rural areas a local endemicity was exceptional,

> *the great tendency of the infection to spread to hitherto plague free localities creates a condition of what might be termed area wide endemicity characterized by marked changes in the localization and extent of the individual outbreaks from season to season*

Several workers besides those in India and China apparently referred to the existence of such an 'area wide' endemicity Thus, as stated by observers such as Robic[7] and Sorel,[8] plague in an 'endemo epidemic form is widely spread on the Madagascan plateau where, as stated by Girard at the second session of the WHO Expert Committee on Plague even in the city of Tananarive the rats continue to be susceptible to the infection Discussing the peculiarities of plague in the Belgian Congo Devignat[15] upheld that in the Ituri region the infection was not immobilized but constantly moved from place to place in a fairly large territory, causing only isolated human cases, which were unconnected with one another in time and space

To judge from statements made by Villafañe Lastra et al[91] and by Macchiavello,[47] an area wide endemicity existed also in some of the South American plague foci

In a valuable study on the spread of plague in the southern and central divisions of Bombay State Sharif[71] reached the important conclusion that two types of epizootic could be observed in these regions In the warm tablelands and plains the infection was often severe, leading to a heavy rat mortality and consequently, to the disappearance of the disease within a short time On the other hand, in the cooler regions comprising the water sheds of the Western Ghats, plague spread slowly but, owing to a lower rat mortality, persisted for a long time In the opinion of Sharif, these areas, which have a moderately moist and cool climate throughout most of the year and also the hilly part of Hyderabad State were endemic centres responsible for the occasional appearance of epidemic plague in the other parts of the affected area of Bombay State

Since, according to the observations of previous workers who have been quoted above, an area wide endemicity seems to have existed in districts of Bombay State now found by Sharif to suffer from occasionally imported epidemics only, one might postulate that the establishment of endemic foci in the hilly parts of the State represents the ultimate stage in the progress of the infection from the coast to the hinterland

Be this as it may it is certain that in marked contrast to urban plague rural plague because it is usually unable to cause a gradual extinction of the susceptible host populations, is not a self limiting disease but is apt to last for very long periods It is important to stress this point particularly because some observers, such as, recently, Baltazard et al[2] believe that in contrast to plague manifestations caused by certain wild rodent species rat caused plague manifestations are invariably of a rather ephemeral

character It may be claimed that the evidence adduced above does not support this view

As stated earlier wild rodents of peridomestic habits may take part in the causation of rural plague manifestations as well as the commensal rats Macchiavello[4] distinguished accordingly between two types of rural plague in South America namely

(1) Pure or campestral rural plague in which *R rattus* and *X cheopis* alone played a role, and

(2) Agrestial rural plague (peste rural agreste) in which epizootics among peridomestic wild rodents were added as a temporary and transitory epiphenomenon to those among *R rattus*

Outbreaks of the former type were far more persistent than the manifestations of agrestial plague because the fleas of the peridomestic rodents were inefficient vectors and the climatic conditions in the open fields were favourable for *X cheopis* at certain times only However as observed in Argentina for instance the presence of agrestial plague was apt to lead to an entrenchment of the infection among sylvatic rodent species

As has been discussed in chapter 7 Roberts [70, 71] found that *X brasiliensis* was the principal vector of *P pestis* in the rural areas of Kenya where plague was endemic in type whereas *X cheopis* was mainly or even solely involved in the urban manifestations of the disease which were of an epidemic character Similarly it was maintained by Sharif & Narasimham [6] that *X brasiliensis* was the main vector in the endemic areas situated in the Western Ghats of Bombay State where plague showed a marked tendency to spread slowly but to persist On the contrary *X cheopis* appeared to play the principal role in the low and warm tablelands of the State where the outbreaks were of an explosive nature but did not last long

In view of the fact that *X brasiliensis* is at least as efficient a plague vector as *X cheopis* these different roles of the two flea species cannot be ascribed to differences in their vector capacity As pointed out by Roberts [70, 71] *X brasiliensis* because it infested mainly the rats which sheltered in the thatched roofs of houses was bound to prevail in the rural areas of Kenya whereas *X cheopis* which mainly infested the rats living underground found a suitable habitat in the towns There can be no doubt as well that the climatic conditions in the hilly districts of Bombay State were more favourable to *X brasiliensis* than those in the tablelands whereas the more adaptable *X cheopis* could thrive in the latter

Sylvatic plague

As aptly stated by writers such as Lobo & Silvetti[4] the fundamental epidemiological difference between rat-caused and wild rodent plague

is that the presence of the infection among the rats is apt to lead to the appearance of collective human cases in settlements whereas wild rodent plague in the strict sense is, as a rule, responsible merely for the occurrence of sporadic cases in persons who have entered the haunts of the species concerned Nevertheless, in view of the often enormous extent of the wild rodent plague foci, the aggregate number of human infections contracted in them may be considerable, and the case mortality is apt to be high since the patients often receive no adequate treatment, either because they live away from centres of civilization or because, owing to its sporadic incidence the presence of the disease is not recognized It must also be kept in mind that importations of the infection from the wild rodent plague foci in Transbaikalia or Mongolia into hitherto unaffected areas of China have led to the most disastrous pneumonic plague outbreaks on record in modern times

Periodicity of Outbreaks

Cyclical periodicity

Since the severity of zootic plague outbreaks in a given locality depends largely on the number of susceptible rodents available, one would expect that a marked depletion of this fuel for the spread of the infection through a severe initial epizootic would lead to a lesser incidence of the disease until the rodents had become numerous once more In other words one might assume a priori that, unless particularly suitable conditions for a rapid re establishment of the rodent population exist (as may be the case in urban communities), zootic plague would show a cyclical periodicity, years in which a severe outbreak occurred being followed by a period of one or two years during which the infection causes less havoc

In the course of an investigation on plague in the Punjab, Greenwood [31] found that this surmise was only in part justified by statistical evidence There was no marked regularity in the succession of severe and mild out breaks and therefore it would not be safe to predict that a province seriously ravaged in one year will escape lightly in the following season He admitted nevertheless, that an exhaustion of susceptible rats exerted some, possibly a considerable, influence on the trend of plague, while climatic changes played a less, probably a much less, important role in this respect Still in Greenwood s opinion, there remained a *tertium quid* which is not apt to be placed in evidence by statistical inquiries based on existing data "

However, observations made by subsequent workers have shown that in India, as well as in other areas, plague outbreaks may show a marked cyclical periodicity Thus Sharif [74] stated that

In the non-endemic areas [of Bombay State] such as are found in the Sholapur District few localities get infected in one plague season and then a very large number

of them in the next one or two plague seasons In the third or fourth plague season only a few of them suffer and then plague disappears for about a year or two

Hence as maintained by Sharif, the peak year in the Sholapur District appears to recur about every five years with complete absence of plague for a year or two

Dealing with the epidemiology of plague in north east Brazil Macchiavello[48] stated

"It is believed that an area in which plague has died out because of a lack of susceptible material may be reinfected if the rodent population happens to become sufficiently great at the same time that an opportunity for reintroduction of the plague organism occurs such as the migration of infected rats If this happens however at a time when one of the periodic nonplague epizootics has practically wiped out the rodent population reinfection will not occur but may skip several years until another opportunity arrives This would explain the reappearance of plague in 5 or 10 year cycles in certain areas

Silva[9] studying the situation in Ceara State in particular, found that a reactivation of plague foci which had been dormant for two to five years was not infrequent

Plague manifestations caused by wild rodents in Argentina showed according to Barrera[5] a cyclical periodicity of two years

In South Africa Davis[1] found

"A periodicity of 5 6 years in the incidence of human plague points to the existence of a general periodicity in the fluctuations in numbers of the wild rodent population in the Union as a whole this shows signs of breaking down as human outbreaks become more and more associated with certain limited hyperenzootic areas

Quite possibly the seriousness of the plague situation existing in the Punjab at the time during which Greenwood made his statistical investigations was in essential respects comparable to that present in the hyperenzootic areas of South Africa

Secular periodicity

Ample evidence has been furnished in chapter I to prove that the plague pandemics following the same laws as individual outbreaks of the disease showed a well marked secular periodicity a stage of gradual rise and spread was followed by a period of full evolution during which the disease because it appeared mainly in the form of major epidemics exacted a grievous toll in lives and this period was followed in turn by a stage of gradual decline The information supplied in that chapter also leaves no room for doubt that the present plague pandemic has reached the period of decline

However even though plague which but a few decades ago ranked high among the diseases decimating mankind now occupies a rather inconspicuous place in the fatality lists it would be wrong to assume that this infection has altogether lost its sting

Indeed reconsidering this problem in the light of what has been discussed in the foregoing pages, one is led to ascribe the great reduction in the incidence of plague largely to the fact that this disease which earlier in the present pandemic raged in urban communities has now almost disappeared from these centres of population, and mainly occurs in rural areas where, as a rule, the case incidence is rather low It follows that the spectacular decrease in the incidence of human plague is not by any means accompanied by a corresponding restriction of the areas in which the infection is present among the rodents On the contrary, it must be realized that the extent of the areas in which the rodents are affected has become greatly enlarged during the present pandemic, because progressing almost invariably from newly invaded coastal regions to the hinterland, plague has become entrenched among the rodent populations, and not rarely among the wild rodent populations, of vast areas which had been unaffected before onset of the pandemic This is a situation which cannot be viewed with equanimity the less so because there is reason to believe that, in some areas, plague among rural rodents, particularly among the wild species, has not yet reached the limits of its possible regional extent, and because it is certain that some of the foci of wild rodent plague are much larger than is usually assumed

Forecasting of Epidemics

Attempts to forecast the appearance of bubonic plague epidemics have been made in various ways

As noted previously, Kunhardt & Chitre [39] proposed a scheme for predicting the reappearance of outbreaks due to a carry over of the infection from the preceding plague season However, Strickland,[83] while approving of this method, emphasized that the formulas worked out for this purpose had only local validity

Forster (quoted by Wu Lien teh [98]) while admitting that in the Punjab a drop in the plague mortality during November and December indicated a slight or at most a moderate, incidence in the following spring, stressed also the importance of climatic factors Prolonged periods without rain during autumn and winter, because they adversely affected the reproduction of fleas were apt to reduce the plague mortality in the following spring The absence of rain during November in particular seemed of great importance in this respect

Comparing the plague statistics for different areas of India with the corresponding meteorological data, Rogers [73] found that seasonal variations in mean temperature and saturation deficiency exerted an important influence on the seasonal plague incidence obviously by acting on the vector fleas Rogers established, in particular, that the climatic factors of the previous year influencing the incidence of the disease were (*a*) the mean

temperature during the hot weather and monsoon seasons and (*b*) the saturation deficiencies in both these seasons as well as in November and December If these values were low plague incidence was favoured However as was to be expected Rogers noted that the effect of favourable climatic influences was less marked after years of high plague incidence than after a period of low incidence of the disease

Rogers found it possible to use such meteorological observations as a basis for forecasting plague epidemics in certain parts of India and obtained as stated by Wu Lien teh [93] satisfactory results during the period 1930 2 However no further advantage seems to have been taken of this method

Convenient though it would be it is not possible to base forecasts of plague epidemics upon observations of seasonal changes in the incidence of the vector fleas It is true that as stated in chapter 7 in regions where X *cheopis* is the sole important vector plague does not assume epidemic proportions as long as the *cheopis* index remains below one and that in localities where the index is constantly above this critical level seasonal changes in the frequency of X *cheopis* are often observable which may be of value in assessing the plague situation It had to be stated however that periods during which plague epidemics occur need not necessarily coincide with those during which the *cheopis* incidence is highest and that indeed it is a high incidence of actually infective fleas and not the frequency of potentially dangerous vector species which is of paramount importance in the spread of flea borne plague

As discussed in chapter 4 Shih & Pollitzer [77] found that observations on the frequency of rats showing signs suggestive of plague at autopsy and/or marked bacteriological evidence of the infection were apt to serve as a yard stick for assessing the seriousness of the plague situation in the localities in question The value of this simple method which gave satisfactory results even when only a limited number of animals was available for examination should not be underrated

Race, Age, Sex, and Occupational Incidence

Most workers are agreed that differences in the race and sex incidence of bubonic plague cases as well as the occasionally observed increased incidence of the disease among certain occupational groups (e g dock workers handling grain cargoes) are due merely to differences in the degree of exposure of the various groups to the infection and not to intrinsic causes The validity of this opinion is well illustrated by observations on the sex incidence of the disease In some plague areas for instance according to Norman White [93] in India females were found to be more frequently affected in others in South Manchuria for example males were more frequently affected while in a third group of foci the incidence of disease

Indeed reconsidering this problem in the light of what has been discussed in the foregoing pages one is led to ascribe the great reduction in the incidence of plague largely to the fact that this disease which earlier in the present pandemic raged in urban communities has now almost disappeared from these centres of population and mainly occurs in rural areas where, as a rule the case incidence is rather low It follows that the spectacular decrease in the incidence of human plague is not by any means accompanied b[illegible]rresponding restriction of the areas in which the infection is present [illegible] rodents On the contrary, it must be realized that the extent of [illegible]hich the rodents are affected has become greatly enlarged [illegible]ent pandemic, because, progressing almost invariably from [illegible]d coastal regions to the hinterland, plague has become [illegible]mong the rodent populations and not rarely among the wild [illegible]tors of vast areas which had been unaffected before onset [illegible] This is a situation which cannot be viewed with equani[illegible] so because there is reason to believe that, in some areas, [illegible]g rural rodents particularly among the wild species has not [illegible] the limits of its possible regional extent and because it is [illegible] some of the foci of wild rodent plague are much larger than [illegible]ly assumed

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temperature during the hot weather and monsoon seasons, and (*b*) the saturation deficiencies in both these seasons as well as in November and December. If these values were low, plague incidence was favoured. However, as was to be expected, Rogers noted that the effect of favourable climatic influences was less marked after years of high plague incidence than after a period of low incidence of the disease.

Rogers found it possible to use such meteorological observations as a basis for forecasting plague epidemics in certain parts of India and obtained, as stated by Wu Lien-teh,[89] satisfactory results during the period 1930-2. However, no further advantage seems to have been taken of this method.

Convenient though it would be, it is not possible to base forecasts of plague epidemics upon observations of seasonal changes in the incidence of the vector fleas. It is true that, as stated in chapter 7, in regions where *X. cheopis* is the sole important vector, plague does not assume epidemic proportions as long as the *cheopis* index remains below one, and that in localities where the index is constantly above this critical level, seasonal changes in the frequency of *X. cheopis* are often observable which may be of value in assessing the plague situation. It had to be stated, however, that periods during which plague epidemics occur need not necessarily coincide with those during which the *cheopis* incidence is highest and that indeed it is a high incidence of actually infective fleas and not the frequency of potentially dangerous vector species which is of paramount importance in the spread of flea-borne plague.

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Forster (quoted by Wu Lien teh [98]), while admitting that in the Punjab a drop in the plague mortality during November and December indicated a slight, or at most a moderate, incidence in the following spring stressed also the importance of climatic factors Prolonged periods without rain during autumn and winter because they adversely affected the reproduction of fleas, were apt to reduce the plague mortality in the following spring The absence of rain during November in particular seemed of great importance in this respect

Comparing the plague statistics for [illegible] of India with the
corresponding meteorological data R[illegible] seasonal variations
in mean temperature [illegible] an important
influence on the sea[illegible] by act[illegible]
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temperature during the hot weather and monsoon seasons, and (*b*) the saturation deficiencies in both these seasons as well as in November and December If these values were low plague incidence was favoured However as was to be expected Rogers noted that the effect of favourable climatic influences was less marked after years of high plague incidence than after a period of low incidence of the disease

Rogers found it possible to use such meteorological observations as a basis for forecasting plague epidemics in certain parts of India and obtained as stated by Wu Lien teh[98] satisfactory results during the period 1930 2 However no further advantage seems to have been taken of this method

Convenient though it would be it is not possible to base forecasts of plague epidemics upon observations of seasonal changes in the incidence of the vector fleas It is true that is stated in chapter 7 in regions where X *cheopis* is the sole important vector plague does not assume epidemic proportions as long as the *cheopis* index remains below one and that in localities where the index is constantly above this critical level seasonal changes in the frequency of X *cheopis* are often observable which may be of value in assessing the plague situation It had to be stated however that periods during which plague epidemics occur need not necessarily coincide with those during which the *cheopis* incidence is highest and that indeed it is a high incidence of actually infective fleas and not the frequency of potentially dangerous vector species which is of paramount importance in the spread of flea borne plague

As discussed in chapter 4 Shih & Pollitzer[77] found that observations on the frequency of rats showing signs suggestive of plague at autopsy and/or marked bacteriological evidence of the infection were apt to serve *as a yard stick* for assessing the seriousness of the plague situation in the localities in question The value of this simple method which gave satisfactory results even when only a limited number of animals was available for examination should not be underrated

Race, Age, Sex, and Occupational Incidence

Most workers are agreed that differences in the race and sex incidence of bubonic plague cases as well is the occasionally observed increased incidence of the disease among certain occupational groups (e g dock workers handling grain cargoes) are due merely to differences in the degree of exposure of the various groups to the infection and not to intrinsic causes The validity of this opinion is well illustrated by observations on the sex incidence of the disease In some plague areas for instance according to Norman White[93] in India females were found to be more frequently affected in others in South Manchuria for example males were more frequently affected while in a third group of foci the incidence of disease

Indeed, reconsidering this problem in the light of what has been discussed in the foregoing pages, one is led to ascribe the great reduction in the incidence of plague largely to the fact that this disease, which earlier in the present pandemic raged in urban communities has now almost disappeared from these centres of population and mainly occurs in rural areas where, as a rule, the case incidence is rather low It follows that the spectacular decrease in the incidence of human plague is not by any means accompanied by a corresponding restriction of the areas in which the infection is present among the rodents On the contrary, it must be realized that the extent of the areas in which the rodents are affected has become greatly enlarged during the present pandemic, because, progressing almost invariably from newly invaded coastal regions to the hinterland plague has become entrenched among the rodent populations, and not rarely among the wild rodent populations of vast areas which had been unaffected before onset of the pandemic This is a situation which cannot be viewed with equanimity, the less so because there is reason to believe that, in some areas, *plague among rural rodents, particularly among the wild species* has not yet reached the limits of its possible regional extent and because it is certain that some of the foci of wild rodent plague are much larger than is usually assumed

Forecasting of Epidemics

Attempts to forecast the appearance of bubonic plague epidemics have been made in various ways

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Comparing the plague statistics for different areas of India with the corresponding meteorological data, Rogers [73] found that seasonal variations in mean temperature and saturation deficiency exerted an important influence on the seasonal plague incidence obviously by acting on the vector fleas Rogers established, in particular that the climatic factors of the previous year influencing the incidence of the disease were (*a*) the mean

temperature during the hot weather and monsoon seasons and (b) the saturation deficiencies in both these seasons as well as in November and December If these values were low plague incidence was favoured However, as was to be expected Rogers noted that the effect of favourable climatic influences was less marked after years of high plague incidence than after a period of low incidence of the disease

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in the two sexes was about the same Landauer[4] found that while this was the case in the towns of Hainan, in the rural areas of that island more females than males fell victims to the infection

Gill [5] maintained that the often observed rarity or even absence of bubonic plague in young children was due to a state of resistance to the infection, engendered through the secretion of an endocrine gland which ceased to function when the children reached the age of about five years However the fact that sometimes cases among young children were comparatively not rare speaks against this assumption For instance, Favarel,[21] in Madagascar, found 104 cases in children up to two years of age out of a total of 2 994 bubonic and septicaemic cases (3 1%)

In the experience of most observers, including Favarel,[21] the incidence of bubonic plague was highest in adolescents and in adults up to the age of about 45 years That this rule is not invariable however is shown by the observations of Barreto & Castro [6] who found that out of 746 plague patients, 724 of whom had bubonic plague, 25 7% were in the age group of 0 9 years and 28 2% in that of 10 19 years

There can be no doubt that this unusual age incidence of the disease in Brazil was due to peculiar extrinsic conditions and not to intrinsic causes

PRIMARY PNEUMONIC (DEMIC) PLAGUE

In opposition to the usual opinion, some workers postulated that primary pneumonic plague might arise de novo, man contracting the infection more or less directly from plague affected rodents without the intervention of human cases of zootic plague with secondary lung involvement

Thus, it was suggested by Simond [80] and by Zabolotny [10] [103] that an introduction of the infection into the mouth by means of fingers soiled either by the faeces of plague infected fleas or by handling plague affected wild rodents might lead to pneumonic plague Connal & Paisley [11] and Nikanoroff [57] maintained that dust contaminated with the faeces of plague infected rodents might also produce a primary lung infection

Scrutinizing the above mentioned and other statements made regarding the existence of 'original' cases of primary pneumonic plague, Wu Lien teh [96] [97] and Wu Lien teh & Pollitzer [100] found that as a rule, such claims had not been substantiated by sufficiently accurate observations The diagnosis was often based merely upon the history of the patients complete autopsies were exceptional and thorough histological investigations had never been made in such cases As proved by observations on tonsillar plague, the fact that oral infection of man with P pestis led as a rule not to primary pneumonic plague, but to bubonic plague often followed by secondary lung involvement also deserved great attention

Nevertheless a number of instances were found which deserved the benefit of the doubt while the originally pneumonic character of a few such cases—due almost invariably to infections contracted in the laboratory—could be taken for granted

As has been stated in chapter 8, one should not be categorical in denying the possibility that patients with so-called primary septicaemic plague may occasionally be instrumental in passing respiratory infection to persons coming in contact with them and the same might hold true exceptionally for patients with uncomplicated tonsillar plague or even for apparently healthy individuals harbouring *P pestis* in their sputum or fauces There can be no doubt however, that in most instances primary pneumonic plague infection is due to contact with patients who suffer from bubonic plague with secondary lung involvement

Rise of Epidemics

The problem of why pneumonic plague epidemics arise received early attention on the part of the Indian Plague Commission [31] who

"noted that there are difficulties in the way of assuming a simple interrelation between the inhalation of plague bacteria in the lungs and the supervention of primary plague pneumonia"

Endeavouring to substitute a more adequate theory the Commission suggested

"(a) that there may be something either in the form or in the manner in which the infectious material escapes from the body which favours the conveyance of the infection into the lungs *of persons in attendance on cases of plague pneumonia (b) that there* may be something specific in the infective material which conditions the supervention of plague pneumonia when the material is introduced into the lungs"

The Commission emphasized that there was no specific difference between the bacilli causing bubonic and pneumonic plague respectively There might be a difference in virulence but the evidence regarding this was contradictory The Commission stressed however that

"the plague bacillus may in the case of infective material derived from a pneumonic case be associated with some other bacillus which favours its growth and contributes to the production of plague pneumonia"

The opinion of the Indian Plague Commission that intrinsic causes are responsible for the rise of pneumonic epidemics has been endorsed by many other workers whose views may be presented as follows

Specific character of the causative organisms

Practically all later observers agreed with the Indian Plague Commission that as far as is known no specific difference exists between strains causing

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Specific character of the causative organisms

Practically all later observers agreed with the Indian Plague Commission that as far as is known no specific difference exists between strains causing

zootic and pneumonic (demic) plague respectively Wu Lien teh[98] aptly stated in this connexion that

it is as easy to cause primary pneumonic plague in suitable animals by inhaling them with a strain from a purely bubonic human case as to produce bubonic plague in laboratory animals by percutaneous or subcutaneous infection with freshly isolated pneumonic strains or even directly with sputum or material obtained at the postmortem of lung victims

This opinion was endorsed by Girard who stated in a recent summary[30] that the oneness (l unicite) of the plague bacillus causing pneumonic as well as bubonic, plague manifestations could no longer be contested

Special virulence of pneumonic strains

The question of whether or not the *P pestis* strains causing primary pneumonic plague are endowed with a particularly high virulence has been answered differently by different workers As will be discussed later some observers are of the opinion that the virulence of the causative organisms increases during the outbreaks but as rightly stated by Wu Lien teh,[98] such an exaltation of the virulence gradually taking place in the course of the epidemics, could not explain their rise

Mixed infection

The opinion of the Indian Plague Commission, that primary pneumonic plague might be the result of a mixed infection has been shared by some subsequent workers

Thus Norman White,[91] on the basis of theoretical considerations conceived the idea that

the plague bacillus *alone* does not and cannot cause widespread epidemics of pneumonic plague and that it seems more than probable that there is an additional organism at work—in other words the plague bacillus in symbiosis with another organism is responsible for epidemic manifestations of pneumonic plague which is a disease *sui generis*

Adopting this idea Nicolle & Gobert[5] expressed the opinion that an association of the influenza virus with *P pestis* might be of importance in the rise of pneumonic plague epidemics A similar conclusion was recently reached by Sokhey,[81] who stated that pneumonic plague was a combined virus and bacterial infection and that the virus factor made the disease highly infectious, whereas plague pneumonias as they occurred as complications to bubonic infection were not infectious

Dealing with the statement of Norman White,[91] Wu Lien teh[98] pointed out with much reason that no line of distinction can be drawn between sporadic and epidemic manifestations of pneumonic plague Whether pneumo pest *spreads* or not depends upon extrinsic and not upon intrinsic factors

In regard to the claim of Nicolle & Gobert [5] who maintained that in Tunis a close connexion existed between outbreaks of influenza and pneumonic plague, Wu Lien teh stated that

"Influenza is not always prevalent at the time of pneumonic epidemics In fact only very few instances are on record where a simultaneous existence of both diseases was noted In many others where special attention was paid to a possible co existence of influenza during pneumonic epidemics the former disease was conspicuous by its absence"

This opinion was endorsed by Girard, [9] who emphasized that in Madagascar the seasonal occurrence of influenza did not provoke an increased incidence of pulmonary complications in the course of plague outbreaks

Contrary to the opinion of Sokhey it is impossible to consider only patients suffering from primary pneumonic plague as infectious in view of the fact that as a rule epidemics of this form of the disease have been caused by bubonic patients with secondary pneumonia It is not surprising that a spread of the infection by the latter patients was less frequent in India than in countries or plague areas with a cooler climate

Pneumotropismus of Pasteurella pestis

As summarized by Wu Lien teh [99] and by Girard [9] some observers tried to explain the peculiarities of pneumonic plague by the assumption that *P pestis* could become specially adapted to the lungs with the result that strains of this nature would produce lung involvement even when entering the body through the skin However the experimental evidence presented in this respect is not convincing and it is also noteworthy that secondary lung manifestations were by no means particularly conspicuous in the few patients who contracted bubonic plague during pneumonic epidemics through contact with the sputum of the sufferers or in other ways e g through direct contact or through the bite of infected human parasites

Moreover it is clear that this supposed pneumotropismus of *P pestis* which could develop only through repeated passage from lung to lung could not be responsible for the rise of pneumonic plague outbreaks

Role of the rodent species involved

Claims were made by some workers that human infections derived from certain wild rodent species especially the Siberian marmot were particularly apt to result in secondary lung involvement and that this peculiarity might account for the frequent appearance of primary pneumonic plague manifestations in the areas in question However the evidence available in this respect is by no means convincing Thus as can be gathered from the

information collected by Wu-Lien teh,[96] the incidence of secondary pneumonic plague was not particularly high in Transbaikalia where the tarabagan was the main reservoir of the infection and commensal rats played no role in the causation of the disease Among 280 plague patients in that area, whose histories could be studied, only 10 might have suffered from primary pneumonic plague

More important still pneumonic plague manifestations have been found to be frequent in countries where ordinary rats alone formed the reservoir of the infection, for instance in Madagascar

Role of the flea species involved

No evidence exists to show that the species of vector fleas involved exerted any influence upon the appearance of pneumonic plague manifestations in man *X cheopis* is the only vector of the infection in Madagascar where pneumonic plague is frequent

It is of interest to note in this connexion that according to Eskey [0] lung involvement was particularly marked in experimental animals which had been inoculated with pooled fleas collected in the USA for the purpose of plague diagnosis, or with fleas infected with *P pestis* in the Plague Laboratory of the US Public Health Service (San Francisco) It would not seem, however, that this peculiar feature was of any practical importance As noted in chapter 1, pneumonic plague was not unusually conspicuous in the USA and most of the cases of wild rodent origin occurred singly

As will be gathered from the statements made in the foregoing pages it has not been possible to ascribe the rise of pneumonic plague epidemics to any peculiar property of the causative organisms or to a mixed infection There is also no convincing evidence to show that the presence of the infection in any particular rodent or flea species is of special importance in the causation of such outbreaks Since, however as aptly stated by Wu Lien teh [99]

> apart from rare instances primary pneumonic plague is not passed directly from rodents to man but arises from human cases with secondary lung involvement it becomes clear that factors which help to mould such secondary pneumonic features deserve our serious consideration

Discussing this problem, Wu Lien teh [98] stressed the fact that as a rule, pneumonic plague outbreaks could be traced back to a single patient —or at most a few patients—suffering from bubonic plague with secondary pneumonia, quite often outbreaks were traced to a traveller who developed such manifestations while proceeding from an infected to a hitherto uninfected locality Clearly, therefore the rise of pneumonic plague epidemics depends not so much upon the frequency with which any kind of lung involvement is present in the bubonic patients in general as upon

the appearance of well marked secondary pneumonia, leading to frequent cough and a copious expectoration of *P. pestis* in individual sufferers.

It has been maintained in this connexion that an unusual susceptibility to respiratory infections in general was apt to influence not only the frequency, but also the severity of secondary pneumonia in bubonic plague patients and consequently the rise of primary pneumonic outbreaks. Thus Girard [2] expressed the opinion that differences in the susceptibility to pneumococcal infections helped to explain why pneumonic plague was rare in the coastal areas of Madagascar and rampant on the high plateau. Similarly Wakil [9] believed that an increased susceptibility of the dark skinned inhabitants of Upper Egypt to lung affections in general was partly responsible for the high incidence of pneumonic plague in that region.

It has to be noted, however, that this factor, although apparently exerting an influence in some plague areas, was found to be of no importance in others. Thus it is noteworthy that the Chinese in Manchuria, while apt to fall an easy prey to pneumonic plague, were rather resistant to pneumococcal infections (Wu Lien-teh [8]).

Factors lessening the resistance to plague infection are no doubt of universal and great importance for the evolution of marked secondary lung involvement in bubonic patients.

Attention was paid to this point by some early workers such as Simond [10] who maintained that plague pneumonia might develop in individuals whose lymph nodes were unable to hold back the causative organism introduced by the bite of infected fleas. A similar view was also put forward in 1905 by Elliot (quoted by Wu Lien-teh [8]).

Far more important than such rather tem[illegible] is the well established fact that travellers who fall ill with bubonic plague after leaving an infected locality or en route are particularly prone to develop marked secondary lung involvement. Petrie & Todd [11] [illegible] that the appearance of these lung manifestations was due to the [illegible] made by such people which, causing the d[illegible] from blood vessels round the buboes, led to [illegible] however, extrinsic factors such as cold or [illegible] nutrition during the journey may [illegible] of marked secondary pneumonia in travellers.

Petrie & Todd [11] postulated that [illegible] particularly vitamin deficiencies [illegible] infection. They assumed that these [illegible] been [illegible] sible for the frequency of pneumonic plague [illegible] as the Black Death.

Sa[illegible] (quoted by Wu Lien-teh [8]) [illegible] n[illegible] travellers [illegible] might develop [illegible] [illegible]

An elaborate hypothesis of Petrie & Todd[61] was that a high saturation deficiency of the air, because it caused an excessive evaporation of moisture from the pulmonary mucous membranes, might lead to the development of secondary pneumonia

Whether differences in racial susceptibility to plague infection might account for the rise of pneumonic plague epidemics, as has been claimed by some observers, seems rather doubtful Presumably, the differences ascribed to this cause were really due to extraneous influences, such as differences in nutrition or other standards of life or to differences in susceptibility to respiratory infections in general

While as shown above, an increased susceptibility to respiratory infections in general, or a lessened resistance to plague infection, is apt to exert an influence on the rise of pneumonic plague epidemics, the appearance of such outbreaks is also greatly promoted by extrinsic factors, such as adverse weather conditions or unfavourable standards of life which lead to close contact between patients suffering from bubonic plague with marked lung-involvement and their families and friends

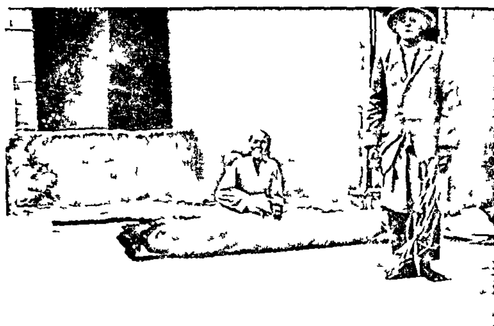
Factors Influencing the Spread of Pneumonic Plague

Infectivity of the patients

Being caused by an entry of *P pestis* through the lower parts of the respiratory tract primary pneumonic plague is almost invariably contracted only by persons coming within close range of the patients and usually results from the spraying of the infective material by the cough of the sufferers Though persons who were near the patients for but a short time occasionally contracted the disease as a rule infection was found to take place in those individuals who had had prolonged contact with the sufferers

Since the frequency of the cough and the quantity of the plague bacilli sprayed by it may show most marked differences, depending upon a greater or lesser severity of the respiratory involvement, it is not surprising to find that the opinions held by different observers in regard to the infectivity of primary pneumonic plague vary greatly It is true that patients in the early stage of the illness i e the stage during which cough and *P pestis* in the expectoration are rare or even absent and similarly patients suffering from "pulmonary" plague without lung consolidation are practically innocuous and that those suffering from slight pneumonic plague, in which cough is often inconspicuous and plague bacilli may be scanty in the sputum are not highly dangerous However, the reverse holds true for patients suffering from the typical form of the disease, in which cough is frequent and the sputum teems with *P pestis* [illegible] it is noteworthy that even the contacts of such patients may [illegible] on w

FIG 33 PNEUMONIC PLAGUE VICTIMS AT MADAGASCAR



Woman wrapping her dead daughter in raffia matting On her back can be seen her grandchild who was found playing near the body of his mother

consciously or unconsciously they adopt precautions which as Wu Lien teh[98] put it may seem useless from a theoretical point of view

Immunity

While as noted in chapter 3 there can be little doubt that instances of natural resistance to pneumonic plague infection exist these are of such rare occurrence as to be of no practical importance The same holds true for the few apparently healthy persons found to harbour virulent *P pestis* in their sputum or fauces who one must suppose have acquired an immunity against the infection How long persons who had been cured of primary pneumonic plague remain immune is still unknown

It has been claimed by some observers that certain races particularly European ones are not liable to contract pneumonic plague That such an apparent resistance to the infection is due merely to extrinsic causes however is well illustrated by the fact that during the 1920-1 epidemic hundreds of Russians living under unfavourable conditions fell victims to the disease

There can also be no doubt that the comparative rarity of pneumonic plague in children and in aged people as well as the sometimes observed

absence of the disease in other groups of the population are due merely to less close contact, or to absence of contact, with patients

Climatic conditions

Though it is generally agreed that the spread of pneumonic plague is most markedly influenced by the prevailing climatic conditions, some differences of opinion exist as to the manner in which these factors exert their influence

Fraser [3] and Jennings [35] maintained in this connexion that an excess of carbon dioxide, likely to be present in overcrowded and ill ventilated houses, might favour the spread of the disease because, as had been shown by Marsh [50] this gas exerted in vitro a favourable influence on the growth and virulence of *P pestis*

According to Manaud [49] lung changes were more marked in plague infected laboratory animals kept at lower temperatures (15° 18°C) than in those kept at about 30°C He also established that guinea pigs which had been made to inhale frozen particles of *P pestis* suspensions were apt to develop primary pneumonic plague, whereas those which had been made to inhale fluid suspensions of the organism at room temperature developed cervical buboes Manaud postulated, therefore, that human infections during the 1910-11 Manchurian outbreak had been effected by the inhalation of frozen sputum particles which, he contended remained more easily suspended in the air and penetrated more deeply into the respiratory tract than liquid particles

Teague & Barber [87] concluded from inhalation experiments made at different temperatures and under varying degrees of humidity that a lower water deficit of the atmosphere, such as is present during cold weather, caused the sputum droplets to float longer in the air—a feature which, in the opinion of the two workers, favoured the spread of pneumonic plague Teague [86] maintained in this connexion that the water deficit in the badly heated houses of Manchuria was not higher than that of the outside air

In the opinion of Chabaneix,[9] the 1910 11 epidemic was probably stopped by the dry weather prevailing in March and April

Scrutinizing this evidence Wu Lien teh [98] pointed out with much reason that pneumonic plague epidemics had occurred not only in cold weather but also in countries with a warmer or even a hot climate where the weather conditions considered as essential by Teague & Barber [87] did not exist and that—far more important still—a mediate infection as presupposed by these two workers and also by Manaud [49] was of rather limited, if any importance as compared to direct infection contracted in the immediate vicinity of the patients

An influence of bad ventilation combined possibly with that of an excess of carbon dioxide in the air, deserves attention but it is not easy to separate

the influence of these factors from that of overcrowding brought about by absolutely or comparatively unfavourable weather conditions such as cool or rainy seasons or marked differences between day and night temperatures

Social conditions

Unfavourable economic conditions or other factors of a social nature which lead to overcrowding play side by side with or sometimes even in place of, adverse weather conditions a most important role in the spread of pneumonic plague The people s habit of congregating round the sick and holding much attended and prolonged burial ceremonies—a common practice in many countries—also exerts an ominous influence in this respect

That adverse social influences may promote the spread of pneumonic plague even in localities with a warm climate is well proved by the observations of Wakil[9] in Upper Egypt He noted that owing to the system used for irrigating the fields in the southern areas of Upper Egypt

"during the month of March April and May when plague is most prevalent in Southern Egypt as well as in the subsequent months great numbers of inhabitants are obliged to remain idle in their badly ventilated houses There is thus a greater risk of the propagation both of bubonic and pneumonic plague than in other parts of Egypt"

This overcrowding of the houses was all the more dangerous because people hailing from the regions concerned often went to work in the then infected ports of Egypt but almost invariably went back to their native places when they contracted a disease If they had become plague infected they often arrived with pneumonic plague complications caused by the long and tedious journey from the extreme north to the extreme south of the country

Control measures

One must fully agree with the statement of Wu Lien teh[9] that "there is probably no infectious disease which theoretically is so easy to suppress as lung plague Indeed even in the past when no effective means for abortive treatment existed isolation of the patients combined with segregation and careful observation of their contacts was bound to cut short the outbreaks Unfortunately however the people resenting the hospitalization of the patients and still more their own segregation [illegible] did all they could to hide the presence of the disease

It has to be noted on the other hand that in certain [illegible] (e g Mongolia) the people themselves often devised surp[illegible] methods of protection against pneumonic plague

Con lu i g remarl s

As will be gathered from the fore[illegible] the spread of pneumoni[illegible]

FIG 34 PLAGUE SERVICE TEAM REMOVING PNEUMONIC PLAGUE VICTIM AT MADAGASCAR



being apt to vary from locality to locality If adequate facilities for control work are available, outbreaks of this disease may be quickly terminated or may even be nipped in the bud Failing such facilities, the infection is apt to spread, particularly if—or as long as—cases of a highly infectious character prevail and if untoward weather conditions or adverse social conditions or, as is frequently the case, a combination of both these factors lead to overcrowding

Local and Extramural Spread

The local spread of pneumonic plague is usually of a familial character Appearing first in one or at most, a few households the infection is carried by visiting relatives or friends to other houses in the same community which are then apt to become subsidiary centres for the spread of the disease A transport of the infection per saltum often takes place simultaneously, effected by persons who are incubating pneumonic plague or who are already actually ill As is well illustrated by a comparative study of the pneumonic plague epidemics in Mongolia and China, the intensity and rapidity of this extramural spread of the infection depend upon the density of the population and the kind of communications available In Mongolia, which was sparsely populated and in Shansi, where the means of communication were primitive

a slow spread took place A much more rapid spread, not rarely characterized by long distance sprints of the infection could be observed in Manchuria which possessed a railway system

Rat Plague during Pneumonic Outbreaks

Dealing with plague in the Punjab, Gill stated that the mode of spread of the pneumonic type is direct from man to man but owing to the readiness with which rats become infected it is liable to give rise to a rat epizootic which in turn gives rise to a bubonic plague epidemic

The validity of this statement which stands in marked contrast to the opinion reached by most other workers appears to be rather questionable if it is considered that (*a*) at the time when Gill made his observations rat caused bubonic as well as pneumonic epidemics occurred in the Punjab and (*b*) it is often rather difficult to prove the persistence of rat plague during the off seasons It seems most likely therefore that the appearance of epizootics during pneumonic outbreaks as observed by Gill was due to the recrudescence of pre existing enzootics and not to a recent spread of the infection from pneumonic patients to the rats

For similar reasons no credence can be given to the claim of Allain [1] that the rat epizootic leading to the 1920-1 plague outbreak in Madagascar was due to the infection of a few rats in the vicinity of a hospital where some earlier pneumonic plague patients had been confined Girard [27] was rather disinclined to agree with Allain s contention

As proved by observations made during the 1910 11 and the 1920 21 epidemics in Harbin and in Vladivostock respectively in very rare instances rats may contract plague during pneumonic epidemics It must be emphasized however, that this transition of the infection never led to the appearance of epizootics It seems altogether unlikely therefore that rats even if they should become infected during such epidemics could prove dangerous

Occurrence of Bubonic Cases in Pneumonic Plague Epidemics

As noted before rare bubonic cases which were definitely not due to an infection derived from plague affected rodents have been observed in the course of pneumonic epidemics According to their pathogenesis or probable pathogenesis these cases may be classified as follows

(*a*) *Infection through the bite of a patient* This unique instance recorded by Leumann [44] concerned a hospital employee in India who had been bitten in the thumb by a delirious pneumonic plague patient The employee developed an axillary bubo on the corresponding side but recovered

(*b*) *Skin infection through pneumonic plague sputum* One instance of this kind observed by Jettmar [36] concerned a woman who having wiped

away the sputum of a pneumonic plague patient, developed a cubital bubo and died

(c) *Entry of plague sputum into the eye* as referred to in the eighth of these studies[69]

(d) *Contact infection through the skin* Two cases of this kind are on record, concerning patients who had kissed pneumonic plague victims and, developing plague carbuncles on the face, succumbed to the infection (Wu Lien-teh[9b])

(e) *Contact infection through the oral or faucial mucosa* The three patients whose records Wu Lien teh[96] was able to obtain showed tonsillar' plague and cervical buboes One of them recovered

Reference has also to be made to a case observed by Fimayer ' The patient in question who had contracted infection through contact with pneumonic plague patients, developed a submaxillary bubo It was considered uncertain whether the infection had entered through the oral or faucial mucosa, or through the eye

(f) *Infections apparently due to the bites of human parasites* One must agree with Wu Lien teh[96] that the few patients with groin buboes observed during the Manchurian pneumonic epidemics had been infected through the bite of human parasites As stated by this author,[99] the rarity of such cases proved " that rat fleas alone are of practical importance in the transmission of bubonic plague '

Age, Sex, and Occupational Incidence

Statistics collected during the 1920 1 epidemic at Harbin[10] showed the following age incidence in 1 252 patients suffering from primary pneumonic plague

Age (years)	*Number of cases*
0 10	21
11 20	83
21 30	593
31 40	385
41 50	130
51 60	33
61 70	6
71 80	1
Total	1 252

It should be noted that the incidence of the disease was highest (78 1%) in the age group of from 21 to 40 years Cases among young children and aged people were rare but, as noted before *it is certain that this was due* merely to lessened chances of contact and not to intrinsic causes

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As summarized by Wu Lien teh et al in 1924,[99] similar observations had also been made during the pneumonic plague outbreak in 1921 at Vladivostock There the incidence of ' septicaemic plague cases, as the Russian workers called cases showing no evidence of lung consolidation was not high during the course of the epidemic, attaining a possible maximum of 15 2% However, except for a few bubonic patients and some doubtful ones supposed to have suffered from intestinal plague, practically all victims seen at the end of the outbreak showed features of " pulmonary ' plague

Since the patients suffering from this type of the disease were obviously little capable or incapable of producing respiratory infection in their contacts, Wu Lien teh and his colleagues felt entitled to assume that the prevalence of such cases at the end of the outbreaks at Harbin and Vladivostock was at least partly responsible for the termination of these epidemics

That pneumonic plague epidemics may be really self limiting was proved by a further observation of Pollitzer & Li [69] in Hunan Province, China, where such an outbreak was seen to end before any control measures had been taken—obviously because the last patients in each of the two first affected households and all patients in the subsequently affected families, had no sputum and in four out of nine instances also no cough The spread of the infection in this epidemic which was due to an importation of the disease by a traveller, may be illustrated thus

Source of infection	*Number of cases*
Contact with patients having cough and bloody sputum	13
Contact with patients having only cough but no sputum	2
Contact with patients having neither cough nor sputum	0
Total	15

The reason why such pulmonary cases become prevalent at the end of the outbreaks is not clear In view of the rapidity with which the change from the pneumonic to the pulmonary form took place in the epidemic observed by Pollitzer & Li,[69] it is difficult to believe that this transition is due to an increased virulence of the causative organisms, produced through their direct passage from man to man It is hoped that further work will throw light upon this interesting and important problem

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Chapter 10

CONTROL AND PREVENTION

ANTI RODENT MEASURES

Control of Commensal Rodents

Killing by mechanical means

The method of killing rats with the aid of sticks or clubs may be advantageous under special circumstances for instance in order to deal with animals met with when digging out burrows, or when otherwise carrying out harbourage demolition However since numerous rats are sometimes met with in the course of such operations some of the animals are bound to escape unless many helpers are available or unless—as was sometimes successfully carried out in China in the case of smaller structures—the buildings in question are temporarily surrounded by a fence made of corrugated iron sheets

Shooting rats—those prowling about at night on garbage dumps for example—has been occasionally resorted to but as the handbook entitled *Rat borne disease prevention and control* [17] aptly points out this method though providing good sport does not materially reduce the number of animals Quite satisfactory results may be obtained when means of flooding rat burrows are available the rats either drowning or being forced to come out and be killed However while this or other methods of destroying rats by simple means may prove advantageous under suitable conditions such procedures are of little if any value in large scale anti rat campaigns It is significant that reference to extensive killing of rats with the aid of sticks seems to have been made in but one recent report (Palestine 1941 [14])

Wholesale killing of rats by the people during plague outbreaks should not be encouraged If they meet with sick rodents at such times they should kill the animals by burning them or by drowning them in some large vessel filled with water Such rodents as well as those found dead during the outbreaks should not be touched directly but should be handled with tongs

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or similar implements which are often available for tending the kitchen fires Pending delivery to the staff, the carcasses should be kept in covered containers

Whether or not the people should be urged to kill rats during the off seasons or in plague free localities in general, depends upon the local conditions Good results have sometimes been obtained in this direction when premiums have been offered for each rat delivered However, it is often difficult to pay such rewards for prolonged periods Generally speaking it is preferable, therefore, to obtain the voluntary co operation of the people in anti rat activities through public health education and propaganda However as will be discussed later, in such educational and propaganda work the attention of the people should be drawn to other methods of rat control rather than to the killing of the animals by mechanical means which often cannot be done on a really effective scale

Use of cats or other predators

Cats

Most modern observers are of the opinion that cats, while useful in keeping the houses free from commensal mice, are of questionable value for the purposes of rat control, particularly for that of *Rattus norvegicus* In order to determine the predatory relation of Baltimore alley cats to Norway rats, Jackson[98] examined 500 specimens of cat faeces and found that rat remains were present in but 6 7% of the specimens garbage or table scraps evidently constituting the staple food of the cats In his opinion the predation of these cats constituted only about 20 per cent of the annual post weaning mortality necessary to keep the rat population stationary However, Storer[21] thought that cats, though in general of minor value in rat control, might be capable of capturing rats which immigrated into premises previously disinfested by other means In the opinion of Barnett,[9] cats like other predators were of some importance in localities where little or no systematic rat control work was done

Dogs

Some, though not all, breeds of dogs particularly terriers, are capable of dealing successfully even with Norway rats Storer[21] maintained in this connexion that rat terriers were particularly useful in dealing with rats which had been blocked (see page 527) or with those trying to escape when stored goods were moved

The large scale use of dogs for rat control was recommended recently by Silva[199] who suggested that suitable breeds should be reared in colonies It is certainly an advantage that, in contrast to cats dogs are little susceptible to plague They, like the cats, do not attack shrews (Herivaux & Toumanoff[8])

Ferrets

Domesticated ferrets have been used for the purposes of rat control in several areas The females of this species are particularly useful in this respect because in contrast to the bigger males they are able to follow the rats into their burrows However as noted in chapter 6 the presence of plague has been confirmed in ferrets used for rat catching in South Africa

Mongoose

Doty[47] stated that mongoose (*Herpestes* sp) introduced into Hawaii in 1883 were considered to be of fairly high value for rat control work However as noted in chapter 6 instances of plague have been observed in such animals The fact that they are unable to climb and are apt to destroy poultry as well as rats also detracts from their value in plague control[234]

Trapping

The traps used for commensal rodents usually belong to two of the three categories distinguished by Mason[130] either enclosing traps which imprison the victims without injuring them or killing traps However professional rat eradicators in the USA in particular also make some use of arresting traps supposed to seize the animals without killing them

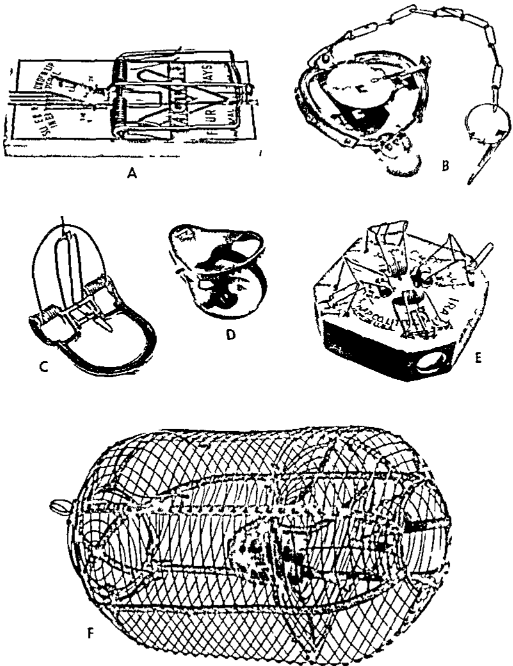
The various patterns of enclosing traps utilized for catching rodents belong either to the class of basket traps similar in shape and construction to those used for catching fish or to the trap door type their doors shutting when a lever upon which the bait is hooked is moved

Trap door traps destined for the capture of individual rats should be made of sufficiently stout wire and should measure about 13 by 6 by $5\frac{1}{2}$ inches (33 by 15 by 13 cm) Basket type traps are often of a larger size so that several rats may be caught successively Usually they are also made of wire but large box like wooden models have been devised by Richter & Emlen[129] for instance and—for the capture of cotton rats—by Scott[139] Suitably small sized enclosing traps must be used for the capture of mice

The generally used patterns of killing traps commonly called snap or break back traps have a solid flat and usually wooden base and kill the animals by the action of a frame made of heavy wire which is actuated by a coil spring and released by a trigger The size of such traps should be about $3\frac{1}{2}$ by 7 inches (9 by 18 cm) for rats and 2 by 4 inches (5 by 10 cm) for mice They can be used unbaited if the trigger surface is enlarged with the aid of a square of cardboard or fly screen so as to provide a platform on which the rodents can step

The arresting steel traps are non baited traps with a platform trigger and two steel jaws which are snapped together by means of a single

FIG 35 TRAPS FOR RATS AND MICE



A Wooden snap or breakback rat trap the same type in smaller sizes is used for mice B Steel jump back trap size 0 (the leather padded jaws are not necessary) C Schuyler trap (made in both large and small sizes) D All steel quickset mouse trap E 4 hole choker loop mouse trap F Wire cage trap (two sizes available)

flat spring The size suitable for rats (No 0) has a jaw spread of about 3½ inches (9 cm) As a rule half or more of the rats are caught alive in such traps [17]

In addition to the above described usual patterns (shown in fig 35) other kinds of traps have been devised including those which kill the rodents by electrocution or with the aid of lethal gases (Nicholson & Vetter [151])

It is sometimes possible to trap many rats infesting a building with the aid of a blocking method The rats must be given food in one of the rooms or cellars of the structure in question which is blocked off so as to possess only one opening communicating with the outside Arrangements must be made by which this opening can be closed from some other part of the building When the rats have become accustomed to frequenting the blocked off room the opening is closed at a time when they are assembled The animals may then be killed with the aid of dogs or by clubbing or—if this can be done safely—a lethal gas may be pumped in

Dealing with the technique of capturing commensal rodents with the traps ordinarily utilized some workers stressed that these ought to be smoked or dipped into boiling water or scrubbed after rodents had been caught in them because otherwise the animals would fear to approach the traps However most recent observers are not in favour of such views Storer [1] for instance stating in this connexion that contrary to popular opinion human odour from handling traps will not keep rats or mice away it is not necessary to wear gloves or to boil wash or smoke the traps

Scalding the traps with boiling water is advisable when rats have been left to decay in them—incidents which should not be permitted to occur If blood or entrails of freshly killed rats adhere to the traps it suffices merely to scrape them off

It is however of decisive importance to keep the traps in good repair and in perfect working condition Those provided with springs must be set on hair trigger , so that the least touch will release the spring As stated in the handbook on rat borne disease [17] a good test of sensitivity is to touch the trigger with a flat piece of writing paper If this is not sufficient to spring the trap the setting is improper or the trap needs adjustment

In order to maintain steel traps in good working condition it is essential to keep them free from rust The technique recommended for this purpose by Wiley [0] is thus summarized in *Rat borne disease prevention and control* [17]

"Pour 1 gallon of water into a crock and then add 2 pounds (1 kg) of phosphoric acid (85 percent H_3PO_4 syrup obtainable from chemical supply companies) Hold rusty traps by their chains and drop them into the solution After about 2 to 3 minutes remove traps and wipe off or hang up to drain

As added in the handbook this procedure removes all rust and provides a protective coating However *badly rusted traps or those treated in pre*

viously used phosphoric acid solutions must be soaked for a longer time

Even traps functioning properly will not prove successful unless they are used in a skilful manner The directions of prime importance in this respect may be set forth thus

1 Commensal rodents will not be attracted by baited traps if other food supplies are available in their vicinity

2 Results of capturing rats may be considerably improved by exposing the baited but unset traps for 3 5 nights so as to give the animals a chance to overcome their fear of new objects Then the traps should be freshly baited and the catches set When trapping mice, it is not necessary to follow this procedure

3 It is also advantageous to camouflage the traps with the aid of pieces of paper, sacking, straw or other materials taking care however, not to interfere with the action of the trigger Satisfactory camouflage can also be effected by placing a board or a box over the traps in such a position that the rats have space to enter and the action of the spring is not impeded

4 If break back traps are exposed on earth floors, they should be dug in so that the top of their base is flush with the ground Some workers also recommend fastening traps exposed on hard floors to some nearby object by means of a cord or light wire about 2 feet (0 5 m) long Break back traps may be fixed to pipes with the aid of hose clamps or may be suspended on overhead beams which are used as runs by the rats As stated in *Rat borne disease prevention and control* [1] for this purpose

> a 1/8 inch [3 mm] hole may be drilled one half inch from the front center of the trap a small finishing nail driven into the beam at the point of setting and the trap hooked over this nail A string from the back of the trap is tied to a nail in the beam below Thus when a rat is caught the trap is jarred off the nail and the rat and trap hang down by the string Traps can be solidly nailed to the beam if desired but these will not permit other rats to travel the same runs so as to be caught in other traps "

5 It is of the utmost importance to put the traps in proper positions As far as possible, traps destined for the capture of rats should not be placed on, but near, the runways, because the animals may be wary of obstructions in their path and also because they like to make little detours in search of food However unbaited snap traps must be placed directly in the runs since they offer no food to lure the animals away In distributing the traps care must be taken to place cage traps with their longitudinal axis parallel to the direction of the runs, and break back traps at right angles to them

The position of the traps should be changed at frequent intervals particularly if no or no more, captures are made

Traps for mice should be placed round their holes or, in the case of a general infestation, round stacks of goods or along walls at regular intervals (Barnett [7])

6 Proper baiting of the traps is as important as their proper placement The opinion that some special sorts of bait (cheese in the case of mice for instance) are particularly attractive to the rodents has sometimes been expressed Actually, however, almost any kind of human food may be used for baiting the traps in many places the rodents showing a preference for the most readily available foodstuffs rather than for choice titbits It is advisable however to vary the baits every few days

7 In order to obtain good results with trapping the traps must be carefully serviced as well as adequately placed and properly baited It is best to distribute them or to set those left in the houses or godowns (warehouses) as late as possible in the afternoon and to inspect them again each morning in order not only to collect the captured rodents but also to reset the traps sprung by rodents which have not been caught Great attention must be paid at the same time to the replacement of baits which have been consumed or have become stale mouldy or rancid Even if the baits have not visibly deteriorated it is usually best to replace them not later than every third day

Since one worker cannot adequately attend to more than 200 traps a considerable staff is necessary for the proper conduct of large scale trapping operations

The views held by modern workers regarding the purposes for which trapping of commensal rodents should be carried out may be set forth thus

(1) General agreement exists that trapping is indispensable for procuring rats and their fleas for laboratory examination in areas invaded or threatened by plague

Most workers recommended the use of enclosing traps for this purpose However, Gross & Bonnet [75] recently postulated that snap traps because they were considerably more efficient in retrieving rodents were preferable to cage traps They admitted that the rats and mice caught in snap-traps lost an appreciable part of their fleas but pointed out that since 2 7 times as many infested rodents were obtained with such traps the total number of fleas collected from these animals was higher than that obtained from the rodents caught in cage traps Since DDT was regularly applied in all human habitations of the plague area in question (Hamakua Hawaii) Gross & Bonnet considered the liberation of fleas from the rodents caught in snap traps as not dangerous Still while admitting that it might be permissible to use such traps in place of cage traps in localities where ample facilities for DDT application are available generally speaking it seems preferable to use the latter for the purpose of obtaining rodents and their fleas for laboratory examination

(2) It is generally held that trapping carried out with a sufficiently large number of traps is usually an effective means of dealing with *mouse*

infestations, the more so because, in contrast to the rats, mice do not become 'trapwise' but continue to be attracted by the traps

(3) Many workers deny that large *rat* infestations can be successfully dealt with by trapping procedures In their opinion rat trapping is merely a method of supplementary value, useful particularly for dealing with small groups of rats left over after poisoning or gassing campaigns or in cases where it is impossible to use poisons or lethal gases

This opinion cannot be considered as absolutely valid because, as shown by some recent experiences, e g, those of Morgan and his collaborators [143] it is possible by waging a kind of 'Blitzkrieg' with very numerous traps to reduce even large rat infestations to proportions manageable by subsequent poison administration It must be admitted however that rat trapping campaigns carried out with the usually available resources, though often continued for a great many years frequently do not lead to a permanent reduction of the rat populations because the numerous survivors, enjoying an increased food supply, multiply freely and thus keep the population level stationary even if many hundreds or a few thousands of these animals are captured every day Hence unless it should prove possible to break this vicious circle by increased trapping other methods must be adopted permanently to reduce the rat populations

Poisoning

The following poisons are used for the eradication of commensal rodents

Arsenic

Arsenic is commonly used for the purposes of rodent poisoning in the form of arsenic trioxide (As_2O_3) often called white arsenic or arsenious oxide sometimes also called arsenious anhydride or arsenious acid It is a white crystalline powder which is rather insoluble in water As a rule therefore it is added to solid baits for the purposes of rodent eradication The toxicity of arsenic trioxide varies according to the size of its particles its acceptibility to the rodents varies according to its purity Therefore as stated by Barnett [9] it is necessary to use a standard, fine refined material which has been tested in the field and found to be satisfactory Kalmbach [102] recommended that arsenic trioxide used for rat poisoning should be pulverized to the point where it would pass through a 200 mesh screen

Statements regarding the concentration at which arsenic trioxide should be added to the bait material vary considerably Workers in the USA [17, 209] recommended a concentration of 3% by weight only, but Dieke [4] recently found that a concentration of 4% was necessary to obtain complete kills of *R norvegicus* under laboratory conditions and baits containing 15% of the poison have been widely used in South America prepared according to Long [117] as follows

(a) Poison packets
( )

Coarsely ground corn meal	35
Cheap wheat flour	35
Grated cheese ground dried fish or meat dried blood or finely ground peanuts	15
Commercial arsenic	15

Put into quantities of one teaspoonful (sufficient to kill one rat) in paper packet

(b) Fish bait
( )

Cheap fresh fish without bones	85
Commercial arsenic	15

Spread as paste on bread paper shavings or banana leaves and placed near rat runs

(c) Fresh blood poison
( )

Fresh blood from slaughter house boiled down to jelly	60
Flour meal or ground salt fish	25
Commercial arsenic	15

Used like the fish bait

The practice in Great Britain (see Barnett [7]) is to mix white arsenic with damp sausage rusk bread mash or soaked wheat in a proportion of 10% per weight (1 part of arsenic in 10 parts of bait) or with sugar meal [a] in the proportion of 15% by weight (1 part of arsenic in 7 parts of bait)

The approximate doses of arsenic trioxide which killed 50% of the animals (LD_{50}) expressed in mg per kg of body weight were according to the handbook on rat borne disease [17] 50 150 in the case of *R norvegicus* and 100 120 in the case of albino rats while doses corresponding to 65 mg per kg of body weight killed all *R rattus* tested (LD_{100}) The poison was also found effective for mice The lethal dose for man is estimated at 1 5 15 mg per kg of body weight so that with a concentration of 3% arsenic trioxide the lethal dose for a man weighing 150 pounds (68 kg) is contained in approximately $^{1}/_{10}$ ounce to 1 ounce (3 30 g) of the poison baits (Ward [9])

Arsenic trioxide has the advantage of being inexpensive As maintained by Long [117] the slow action of this chemical is also advantageous because the poisoned animals succumbing not sooner than 24 hours after consumption of the baits are able to seek refuge in their burrows instead of dying in the open and thus scattering their fleas

On the other hand the fact that arsenic is a highly dangerous poison for domestic animals and man as well as for the rats deserves most serious attention Still the use of 17 691 pounds (8 070 kg) of packets containing arsenic trioxide in Guayaquil Ecuador caused according to Long [117] comparatively few fatalities in domestic animals (mainly in chickens sometimes in dogs or cats occasionally in donkeys) and but one death in man— namely of a boy who had partaken of 10 poison packets The brother of this child who ate 6 of the packets survived The distribution of over 40 000

[a] Consisting of 9 parts by weight of national flour and 1 part of castor or fine sugar

pounds (18,000 kg) of arsenic containing fish baits caused no accidents in man or in domestic animals the latter not being attracted by this type of bait

To minimize accidents, tartar emetic may be added to the poison baits in the proportions indicated in table XIX

TABLE XIX PROPORTIONS OF TARTAR EMETIC TO USE WITH RODENTICIDES

Rodenticide	Small amounts			Large amounts		
	poison	tartar emetic	bait material	poison	tartar emetic	bait material
Arsenic trioxide	60 g	22.5 g	1.917 kg	1 lb (453 g)	6 oz (170 g)	32 lb (14.5 kg)
Thallium sulfate	10 g	6 g	1.9[illegible]4 kg	2 ½ oz (71 g)	1 ½ oz (42 g)	30 lb (13.6 kg)
Zinc phosphide	20 g	7.5 g	1.972 kg	4 oz (113 g)	1 ½ oz (42 g)	25 lb (11.3 kg)
Alphanaphthylthiourea	60 g	60 g	1.880 kg	1 lb (453 g)	1 lb (453 g)	31 lb (14.0 kg)

* As recommended in *Rat borne disease prevention and control* [311] (metric weights have been given in brackets to facilitate comparison)

Persons poisoned with arsenic trioxide (or with other arsenic-containing substances) ought to be treated by stomach lavage and administration of magnesium sulfate as a cathartic If they are seen immediately after they have swallowed the poison the officinal antidote, consisting of a freshly *prepared solution of ferric hydroxide and magnesium oxide,* may be administered first

Sodium arsenite

Although sodium arsenite (Na_2HAsO_3) according to *Paranjothy*[153] in a concentration of 10% is as effective as arsenic trioxide, it has never been much utilized as a rat poison It deserves attention however, that being more soluble in water than white arsenic, it can be used not only in bait form but also as a spray According to the handbook on rat borne *disease* [17] *sodium arsenite solutions prepared for rodent poisoning* should have a concentration not exceeding 3%

Barium carbonate

Barium carbonate ($BaCO_3$) a *heavy fine, white powder, has been found* useful as a rat poison only if added to the bait material in high concentrations—according to most workers in a proportion of 20% by weight *Its* LD_{50} was found to be 750 mg per kg of body weight in the case of albino rats (Barnett[8]) and 1,480 ± 340 mg/kg body weight (Dieke & Richter[43]) in the case of *wild Norway rats* most of which succumbed within 24 hours after

administration of the poison. The lethal dose for man is, according to Ward,[23] 800 mg per kg of body weight, so that with a poison concentration of 20% 9.9 ounces (280 g) of the bait mass are fatal for a man of 150 pounds (68 kg).

Barium carbonate is relatively innocuous to domestic animals except to cats which seem to be particularly susceptible to this poison. This rodenticide also possesses the advantage of being as inexpensive to use as arsenic trioxide (Paranjothy[158]). Nevertheless in the opinion of most recent workers, in view of the availability of more efficient rodenticides barium carbonate should not be used any more. Though this holds true in general, one should not overlook the fairly satisfactory results obtained with this poison by Harrison & Woodville[9] during a recent plague outbreak in the Bahan precinct of Rangoon, Burma.

The procedure adopted by the two workers was

(*a*) to lay out at suitable points from 4 to 8 ounces (100-200 g) of boiled rice

(*b*) to renew this rice daily for 3 more days of prebaiting

(*c*) to replace on the fifth day the baits by a similar amount of boiled rice which contained about 16% (1 part in 5 by weight) of barium carbonate

(*d*) to collect and destroy excess poison baits and dead rodents on the sixth day

While before poisoning the mean number of rodents trapped was 22.4 per night with a standard error of 1.5, after poisoning the mean number trapped was 8.3 ± 2.2, indicating a reduction of about two thirds. The incidence of plague in the area treated dropped accordingly, only three cases being recorded after the poisoning campaign. It is noteworthy that the two rodent species most frequently met with at the time were *R. exulans concolor* (45%) and *Bandicota bengalensis* (31%).

As far as the present writer is aware, the distribution of baits containing barium carbonate never led to fatal accidents in man. Care has to be taken, however, to keep this poison separately and not in the drug stores so as to avoid its being issued instead of barium sulfate which, unlike $BaCO_3$, is insoluble in water and can be ingested with impunity.

In cases of barium carbonate poisoning the stomach must be emptied with the aid of an emetic or a stomach tube, preferably by lavage with water containing one ounce (28 g) of magnesium sulfate per gallon. Sodium sulfate or magnesium sulfate (well diluted) should then be administered as often as necessary in one-ounce doses in addition to symptomatic treatment.

Phosphorus

While but rather limited use has been made of phosphorus-containing baits for large-scale anti-rodent campaigns, this highly dangerous chemical forms the base of widely sold rat and mouse poisons to be used by laymen in the form of spreads on bread or other foodstuffs. This is a practice which should be vigorously discouraged or, better still, altogether prohibited.

Should there be any need for rodent baiting by laymen the now available red squill preparations or, better still, the inoffensive substances mentioned below ought to be preferred

There is also no more reason for continuing the use of phosphorus for general anti rodent campaigns It seems unnecessary, therefore, to give directions here for the utilization of this poison

Red squill

The preparations of squill used for rat poisoning are derived from the dried and ground bulbs of the red variety of *Urginea maritima*, a plant native to the countries around the Mediterranean Their action depends upon the presence of a rodent toxic glycoside scilliroside, which was isolated by Stoll & Renz [11] in 1941

Red squill is no doubt the oldest rodenticide still in use, having been recommended for the eradication of mice by an Arabian author writing early in the 13th century (Stoll & Renz) Shepard [196] even maintained that reference to the rodenticidal properties of red squill had been made on stone tablets dating from the pre Christian era

Red squill also occupies a unique position among the usual rodent poisons in so far as it is rather safe to use because most animals other than rodents refuse to consume baits containing this poison and because it possesses marked emetic properties Consequently it is apt to prove fatal only to those animals which are unable to vomit particularly the rodents and not to man or most domestic animals

Desirable as it is therefore to make large scale use of red squill for the purpose of rodent control, this is difficult in so far as the originally available preparations are apt to vary widely in potency and often prove rather ineffective In order to obtain satisfactory results, it is indispensable therefore to use exclusively brands of red squill of which the efficacy has been ascertained through methods of biological standardization It is of great importance in this connexion that, as found by Crabtree et al,[3] the potency of low grade red squill powders may be brought to a satisfactory standard by extracting the active rat killing principle from them and adding adequate amounts of these extracts to the raw powders to be fortified

Depending upon the kind of rats used for the biological standardization tests the standards recommended by different workers vary A satisfactory recommendation made by Barnett[9] was that red squill preparations should have an LD_{50} of 500 mg/kg for wild Norway rats Similarly a toxicity standard of 600 mg/kg is considered as minimal in *Rat borne disease prevention and control* [17] As stated there, the products available in the USA often proved effective against rats in lower concentrations than the 10% by weight recommended for 500 600 mg/kg red squill Nevertheless it was found desirable to re test each batch before actually purchasing it because such tests on locally obtained rats were bound to indicate not only

the desired concentration of the poison but also the acceptability of the baits The procedure recommended for this purpose was as follows

Rats in separate cages should be given small baits (90 to the pound [0 45 kg]) with proportions of squill to baits of 1 9 1 11 1 13 1 15 1 17 1 19 1 21 and 1 23 by weight If the baits containing 1 21 and stronger kill the rats but the 1 23 does not it is recommended that the next stronger mixture be used in the field that is the 1 19 mixture The kills during tests should occur in about 12 to 24 hours

As maintained by observers such as Doty[47] and Meunier & Rouffia[131] *R rattus* was found to be more resistant to red squill than the Norway rats Meunier & Rouffia noted in this connexion not only that higher doses were necessary to kill *R rattus rattus* and *R r alexandrinus* but that these animals survived for at least 24 hours after poisoning

It is generally held that red squill is unsuitable for poisoning domestic mice However Dybing et al[50] ascribed these failures to a poor acceptance of the baits containing the poison by these animals and not to their lessened susceptibility to red squill Actually the red squill preparations tested experimentally by Dybing et al[50] proved more toxic to mice than to rats

Most observers found that male rats were considerably more resistant to red squill preparations than the females This difference seems to be due to hormonal influences because as shown by Crabtree et al[33] castrated male rats were as susceptible as the females but became once more resistant when given testerone preparations The fact that according to Dybing et al[50] the resistance of young male rats to scilliroside was lower than that of animals weighing over 100 g may serve as corollary to these observations

As stated in the handbook on rat borne disease[217] red squill can be used successfully with all food baits which can be ground finely and mixed thoroughly with the rodenticide Cubed fruit or vegetables are unsatis factory because they will not hold enough squill to kill the rats Unless lower dosages are indicated by locally made standardization tests the poison should be admixed with the bait material in a proportion of 1/9

Large scale tests with a red squill concentrate containing 0 7% scilli roside were carried out by Barnett et al[11] who found that

(*a*) this preparation was effective in the field against *R norvegicus* but not against *R rattus*

(*b*) assayed against white rats it had an LD_{50} of about 25 mg/kg for males and about 5 mg/kg for females

(*c*) the LD_{50} for adult fowls was more than 400 mg/kg while pigs dogs and cats given doses of up to 16 mg/kg did not succumb

(*d*) the preparation was unpalatable to pigs dogs and cats but was consumed by fowls if admixed with mash in a concentration of 1%

Since red squill is extremely irritating to the skin great care must be taken not to handle or even to touch it with bare hands Preferably rubber

gloves should be worn during bait preparation The baits must be distributed or collected with the aid of implements such as forceps tongs chopsticks

Though otherwise red squill is the safest among the rat poisons of the usual type, it is nevertheless necessary to prevent access of children and domestic animals to the baits Particular care is indicated in the case of sheep and goats said to be unable to vomit In cases of squill poisoning the stomach should be emptied unless copious vomiting has occurred and symptomatic treatment should be administered to prevent collapse

Strychnine

Most modern workers, if enumerating strychnine at all among the rodent poisons, are agreed that in view of its poor acceptance by commensal rats it is unsuitable for the control of these species This was for instance, the opinion of Emlen & Stokes[53] who recorded that strychnine sulfate given in 1% concentration to *R norvegicus* killed but 32% of the animals tested Dieke,[49] using this poison in lower strengths found that only 6 out of 20 Norway rats were killed, the lethal dose (LD_{50}) being 4 8±0 4 mg/kg body-weight

However, some workers recommended baiting with alkaloid strychnine or with strychnine sulfate for the control of commensal mice and as will be discussed later as well as for that of certain wild rodent species Thus Moore[140] though considering trapping the method of choice for the control of commensal mice, stated that they might be poisoned with a mixture made from (*a*) 1 ounce (28 g) of powdered strychnine alkaloid (*b*) 2 ounces (56 g) of common borax , and (*c*) 8 pounds (3 6 kg) of large flaked rolled oats, distributed in teaspoonful quantities or exhibited in little boxes so as to provide a permanent poison supply available to the mice at all times

Storer,[12] while admitting that baiting with strychnine coated wheat was useful for mouse control in outbuildings stated that trapping these animals was the better practice in residences Indeed since arsenic trioxide and zinc phosphide are also effective for the control of commensal mice it seems rather questionable whether strychnine should be used at all to poison these animals

The treatment indicated in the case of strychnine poisoning is described in the 1952 edition of *The Extra Pharmacopoeia*[17] thus

"The main object of therapy in strychnine poisoning is the prevention of convulsions and the immediate treatment is the intravenous injection of a short acting barbiturate such as thiopentene sodium Light chloroform anæsthesia may be used temporarily to control convulsions if a barbiturate is not immediately available Gastric lavage may then be carried out using a solution of potassium permanganate 60 gr in 2 gal of water or a 2% solution of tannic acid may be employed The patient should be kept lying down in a darkened room If respiratory depression occurs artificial respiration or inhalation of oxygen with 5% carbon dioxide should be employed

Thallium sulfate

Thallium sulfate ($Tl\ SO_4$) is a heavy white crystalline powder which is fairly soluble in water It has been found fully effective against Norway rats as well as against *R rattus* the lethal dose for the former (LD_{0}) being 15 1 16 7 mg per kg of body weight that for *R rattus* (LD_{100}) being 35 mg/kg [17] Its action is slow the killing time for Norway rats varying in the experience of Dieke & Richter [43] from 1 5 to 6 days

Unfortunately thallium sulfate though one of the most potent rodenticides is also one of the most dangerous the more so as it possesses no distinctive odour or taste is readily absorbable through the unbroken skin, and is apt to cause secondary poisoning in predators like cats and mongoose which devour the carcasses of poisoned rodents (Doty [4]) Dogs are also quite susceptible to this poison (LD_{50} less than 16 mg/kg [217])

The lethal dose for man is according to Danzel [31] about 0 02 g (20 mg) per kg of body weight i e 1 2 1 5 g for an adult 0 5 0 6 g for a child Numerous instances of thallium poisoning including cases of persons consuming bait materials mixed with this chemical are on record (see Munch et al [146] Chodzko [8]) One must therefore fully agree with the now generally accepted opinion that thallium sulfate which is also comparatively expensive should not be used for the purposes of general rat control at least not unless tartar emetic has been added to the bait material (see table XIX page 532) Indeed the present writer for one fails to see why it should be used at all

Thallium sulfate was formerly admixed with the bait materials in proportions of 1 5% or even 2 0% by weight but as shown by the experience of Doty [47] and confirmed by recent tests in the USA, [17] a concentration of 0 5% is sufficient It may be incorporated in the usual solid bait materials or distributed in aqueous solution The use of rubber gloves is indispensable for the preparation of solid or fluid baits and contact with these materials during their distribution or collection must be rigidly avoided

As stated in *The Extra Pharmacopoeia* [1 7]

" Washing out the stomach and use of laxatives are important in acute poisoning [with thallium] Injection of histamine followed by renewed rinsing of the stomach and the use of diuretics is also advisable To render inactive any thallium circulating in the blood 10 ml of a 10 / solution of sodium thiosulphate should be given intravenously This can be repeated several times a day and should be continued over a period of time "

Blood transfusions and administration of large doses of vitamin B_1 and vitamin B_2 may also be advisable (Prick et al [173])

Zinc phosphide

Zinc phosphide (Zn_3P_2) a black powder slightly soluble in water often possesses a garlic like odour due to the slow release of phosphine gas

(PH_3) which, while apt to be repulsive to man and domestic animals, seems to attract rather than to repel the rodents

Zinc phosphide is generally considered to be an efficacious poison for rats and mice, the handbook on rat borne disease [17] for instance considering it more effective against commensal rats than arsenic trioxide or red squill though less so than sodium fluoroacetate and thallium sulfate (see table XX)

TABLE XX RELATIVE EFFECTIVENESS AND SAFETY OF THE MOST COMMON RODENTICIDES *

Rodenticide	Recommended concentration in baits by weight (%)	Relative effectiveness against rats	Relative safety to man and domestic animals
Sodium fluoroacetate	0 32	1	6
Thallium sulfate	0 5	2	5
Zinc phosphide	1 0	3	3
Alphanaphthylthiourea	2 3	4	2
Arsenic trioxide	3	5	4
Red squill (fortified or extract)	5 10	6	1

Data obtained from *Rat borne disease prevention and control* [11]
Recommended only for use in water

The lethal dose of zinc phosphide (LD_{50}) for Norway rats ranges according to Dieke & Richter,[43] between 37 6 and 43 4 mg per kg The LD_{100} for *R rattus* is 42 5 mg per kg [43] as compared to 40 mg/kg in the case of dogs and presumably also in that of man [17] Generally speaking zinc phosphide acts fairly rapidly, killing the rodents in less than 24 hours according to the handbook on rat borne disease [217] *usually in 6 12 hours* Nevertheless, Morgan [141] found that after baiting with this poison the majority of the rats did not die out in the open but under floorboards or behind casings and partitions However, Middleton [139] stated that on farms many rats poisoned with zinc phosphide died in the open

Opinions on to what extent zinc phosphide can be used safely for the control of commensal rodents vary considerably In the handbook on rat borne disease [217] it is admitted that this poison is safer than other highly toxic rodenticides because both its odor and color are objectionable to man and pets ' Nevertheless the view is expressed that, except ' in those business establishments where a toxic poison will be safe zinc phosphide should be distributed only in baits containing tartar emetic as well (see table XIX page 532) Sharing this opinion Emlen & Stokes [53] during a rat poisoning campaign in Baltimore used exclusively zinc phosphide baits to which

tartar emetic had been added in a proportion of 1% However these baits which were considered as "essentially harmless", gave poor results

It must be noted, on the other hand that large scale use of zinc phosphide baits to which no tartar emetic had been added, has been made outside the USA for instance in Hawaii (Doty[4]), in Great Britain (Barnett[7, 9]) and in Italy (Martorana[1, 8]) apparently without any serious mishap Doty who used zinc phosphide in a concentration of 0 5% considered it less dangerous for domestic animals than thallium sulfate and maintained in particular that in contrast to the latter zinc phosphide did not produce secondary poisoning in predators which had devoured poisoned rodents However as was pointed out in a review of Doty s article in the *Bulletin of Hygiene* [0] in Great Britain where higher concentrations of zinc phosphide were used fatalities had been noted in cats after they had fed upon rats killed by this poison According to Middleton s[139] experiences in farms zinc phosphide was dangerous for poultry as well as for cats

As will be gathered from the statements made above different concentrations of zinc phosphide have been used for rat eradication The recommendation in the handbook on rat borne disease[217] was to add this poison to the bait material in a proportion of 1% by weight Lesser proportions were used by some workers such as Doty[47] (0 5%) and Johnson[99] (0 75%) higher ones by Emlen & Stokes[53] (3%) and Martorana[128] (5%) The official British recommendation[7] was to add zinc phosphide to wet bait bases (such as damp sausage rusk bread mash or soaked wheat) in a proportion of 2 5% by weight (i e 1 part in 40 parts of bait) or to admix it with sugar meal in a proportion of 5% by weight (1 part in 20 parts of bait) It should be noted in this connexion that with a concentration of 2% in the baits *the lethal dose of zinc phosphide for a man of 150 pounds (68 kg)* is contained in 4 9 ounces (138 9 g) of the bait material (see table XXI)

TABLE XXI TOXICITY TO MAN OF RODENT BAITS PREPARED WITH COMMON POISONS

Poison	Accepted lethal dose to man (mg/kg)	Concentration used in baits	Weight of bait containing a lethal dose for a 150 lb (68 kg) man	
			(ounces)	(grams)
Arsenic trioxide	151 0	3 (1 33)	0 12 1[illegible]	3 434 6
Barium carbonate	800 0	[illegible]0 (1 [illegible])	9 9	[illegible]06
Strychnine	1 0	0 3 (1 3[illegible]0)	0 8	[illegible]7
Thallium sulfate	20 0	1 5 (1 65)	3 [illegible]	90 6
Zinc phosphide	40 0	[illegible]0 (1 [illegible])	4 9	1[illegible]39
Alphanaphthylthiourea	unknown	5 0 (1 [illegible]0)	Probably very large	
Sodium fluoroacetate	5 0	0 4 (1 [illegible])	3 15	89

After Ward

bait refusal The validity of Emlen s experiences was confirmed through recent laboratory studies of Gaines & Hayes [58] who found that under simulated field conditions bait shyness could be demonstrated for at least four months after the last exposure to ANTU, i e , much longer than had been formerly assumed

Norway rats which have consumed sufficient doses of ANTU as a rule die fairly rapidly, the survival time ranging according to Dieke & Richter [43] from 16 to 30 hours Rats of this species as well as other animals which succumb to acute ANTU poisoning show, at autopsy, marked pulmonary oedema or pleural effusion, or a combination of both Landgrebe & Mor gan [109] maintained in this connexion that pulmonary oedema was massive in rats dying within four hours after ANTU poisoning while those surviving longer showed pleural effusion As noted by McClosky & Smith,[1 3] rats which had been killed by large doses of ANTU after they had become tolerant to the poison showed no pleural effusion even when they died within 24 hours but usually their lungs were oedematous and haemorrhagic The liver of some of these animals and of all rats surviving ANTU poisoning for more than 72 hours showed fatty degeneration

Rats succumbing to ANTU poisoning are apt to die in the open pre sumably because they suffer from air hunger (Violet & Rinaudo [3])

ANTU may be admixed with all sorts of bait materials, including cubed fruits or vegetables However, as pointed out with much reason in the handbook on rat borne disease, [17] under urban conditions, finely ground corn or other sorts of grain, because not usually eaten by cats or dogs form the safest vehicle for the poison The concentration at which ANTU was added to the bait material by the different workers varied from 1% to 3 5% a concentration of 2% 3% as recommended in the handbook on rat borne disease [17] is generally speaking most satisfactory Violet & Rinaudo,[2 3] when recently using ANTU for a large scale poisoning cam paign in Lyons France, wrapped their baits which contained 3 5 % of the poison, in quantities of 4 5 g in newspaper Though they distributed 20 60 of these packets per building each packet containing 140 175 mg of ANTU, they did not encounter any untoward incident

Besides being used in the usual form of baits, ANTU (in a concentration of 20% in pyrophyllite or some other inert material) has been distributed in the form of copious patches on rat runs in rat burrows, or in other har bourages in order to kill the rodents which after having walked through these patches, consume the poison by licking it off from their paws and fur One must, however, agree with the handbook on rat borne disease [17] that the use of this rather expensive procedure should be restricted to localities where it would be inadvisable to distribute ANTU in ordinary baits

A modification of this method was to utilize a mixture containing 8% DDT as well as 20% ANTU and 72% of an inert powder, so as to kill the rat fleas as well as the rats coming in contact with the patches However,

Wiley[31] found that in buildings so treated only 41% of the Norway rats were killed Deaths among the commensal mice amounted to 70% but there can be little doubt that this better result was due to the action of the DDT rather than to that of the ANTU

So far no antidote to ANTU poisoning in man has been found However as pointed out by Richter[177] the marked insolubility of this compound makes prompt voiding of the stomach preferably by lavage an effective counter measure Cathartics and alkalis should not be used in cases of ANTU poisoning Should oedema of the lungs develop one should stop the intake of fluids and administer oxygen[177]

Sodium fluoroacetate

Often called ' 1080 according to the catalogue number of the originally tested sample sodium fluoroacetate was singled out as a most effective rodenticide in the course of large scale screening tests carried out in 1943 and 1944 in the USA (Kalmbach 1945[100] 1948[101]) Most interestingly it was afterwards learnt that monofluoroacetic acid was the toxic principle of a plant (*Chailletia toxicaria*) used to poison rats in Sierra Leone (Klingen smith[107]) and also of *Dichapetalum* (*Chailletia*) *cymosum* commonly called gifblaar feared as a stock poison in South Africa (Marais[126])

Sodium fluoroacetate is a light white crystalline compound which is tasteless and possesses no or at least no marked odour It is easily soluble in water but practically insoluble in oils As shown by the figures quoted below this poison is a most effective rodenticide but unfortunately is at the same time also most dangerous for other animal species and for man

As can be seen in table XXII dogs cats pigs and goats are particularly susceptible to 1080 As pointed out in this connexion in the handbook on rat borne disease[17] a dose of 2 5 mg capable of killing 50% of Norway rats weighing about one pound (0 45 kg) would also kill a dog weighing 25 pounds (11 kg) or a cat weighing 10 pounds (4 5 kg) As far as it is per missible to judge from the experience made in monkeys a 15 pound (7 5 kg) child might be killed by consuming 35 mg of 1080 i e less than the amount (50 mg) contained in a 1/2 ounce (14 g) cup full of 1080 solution as used to poison rats

Symptoms in rats poisoned with sodium fluoroacetate begin to appear after 20 minutes and the animals succumb after 1 8 hours or even sooner (according to Dieke & Richter[43] after 45 240 minutes as compared to 12 90 minutes in the case of strychnine sulfate) The rats obviously because they are rapidly overcome by the action of the poison usually die in the open quite frequently near the places where the poison baits or cups have been exhibited Hence unless stringent precautions are taken instances of secondary poisoning in cats dogs or pigs which devour or even merely gnaw the carcasses of 1080 poisoned rodents or birds are bound to occur

the more so because the carcasses of the poisoned animals remain dangerous even if they have decayed or have become dry The death toll recorded by Gilcreas[63] in an instance where 1080 baits had been placed deep in the rat burrows of a village dump was about 20 dogs and cats

TABLE XXII LD$_{50}$ OF SODIUM FLUOROACETATE EXPRESSED IN MG PER KG BODY WEIGHT*

Animal	LD$_{50}$ (mg/kg)	Animal	LD$_{50}$ (mg/kg)
Norway rat	3.7 (or less[a])	Horse	1.0
R rattus	1.4	Pig	0.3
Albino rat	2.5-7	Chicken	6.30
Commensal mouse	8.10[b]	*Mourning dove (Zenaidura macroura)*	7.10[b, c]
Cat	0.35-0.50	Sparrow (*Passer domesticus*)	2.7[b, d]
Dog	0.07-0.20	Rhesus monkey	5.75
Goat	0.3-0.7	Man (estimated)	2.0

Data obtained from *Rat borne disease prevention and control*[* [illegible]]

[a] The LD$_{50}$ in 55 starving Norway rats poisoned by Dieke & Richter[[illegible]] with the aid of a stomach tube was 0.22 ± 0.01

[b] According to Hughes[[illegible]]

[c] Only 33% of these birds were killed

[d] LD$_{16}$

It is important to note in this connexion that, as proved by Gratch et al[71] (*a*) sodium fluoroacetate does not reach the liver and spleen of rats killed by this poison in sufficient amounts to cause secondary poisoning in guinea pigs inoculated with such tissues for diagnostic purposes and (*b*) 1080 in the concentration used for rat poisoning does not exert a bacteriostatic action on *Pasteurella pestis*

As shown by studies of Chenoweth & Gilman[5] the causes of death in animals poisoned with methyl fluoroacetate were different in different species In the case of rabbits goats horses and spider monkeys the heart became primarily affected and death was due to ventricular fibrillations *In the case of the dog and guinea pig* the central nervous system was primarily affected and death was caused by cessation of the respiratory activity In cats, pigs, and rhesus monkeys both the heart and the central nervous system became involved Rats as well as hamsters developed changes in which depression and delayed bradycardia were prominent but did not usually exhibit ventricular fibrillation Death in these two species if occurring early (4 6 hours after), appeared to be due entirely to respiratory depression

As stated in the handbook on rat borne disease[17] rats surviving the administration of sublethal doses of the poison show neither aversion to

nor serious tolerance of 1080 However Barnett & Spencer[1] found that in five out of six field tests made by them populations of *R norvegicus* which had survived baiting with 1080 showed shyness [refusal] of the poison when it was given in a new bait base The general conclusion reached by these two workers was that although 1080 is probably more effective in direct poisoning than other poisons used in the past it does not give as consistent results as the standard poisons do after prebaiting

Though 1080 may be easily admixed with all sorts of solid bait materials the workers in the USA urged with great reason that this poison should be distributed preferably in fluid form It must be kept in mind in this connexion that the rodents instead of immediately consuming solid poison baits may carry them away to places where they cannot be recovered

As has been shown by Nicholson et al [150] a concentration of 12 g of sodium fluoroacetate per gallon of water is fully effective in killing Norway rats Addition of 7 g of 1080 per gallon of water suffices to kill *R rattus* and mice

Though sometimes advantage has been taken of watering fountains as used in chicken yards to make 1080 solutions available to the rodents as a rule shallow cups able to contain no more than 3/4 of an ounce (21 g) and filled to only half their capacity are utilized for this purpose

Wiley [3] recommended the use of cups made of waxed paper on which were stamped by means of red waterproof ink the word Poison and suitable symbols (e g a skull and cross bones) He also advocated that the cups should be dyed a light brown colour so as to make them less conspicuous to children

As a further precaution it was recommended that a solution of nigrosine be added to the 1080 containing fluids so as to give them a warning black colour The acceptance of the solutions by the rats seemed not to be materially reduced by the addition of this dye [17]

Concentrations of 1080 varying from 0 25% to 1% have been used by different workers for the preparation of solid baits The standard recommendation of Ward [9] was to mix one gram of the poison with each pound (0 45 kg) of the bait material thus using 1 ounce (28 g) for 28 pounds (12 7 kg) of bait

Rubber gloves must be worn by the staff members preparing 1080 solutions or baits The latter must not be touched with bare hands but must be distributed and collected with suitable instruments Unused baits as well as poisoned rodents which must be collected as soon as possible should be disposed of by thorough incineration or by burial at a depth of at least 2 feet (0 75 m) The latter method should also be used when it is impossible or inadvisable to dispose of unused 1080 poisoned water by flushing it down a sewer Preferably the cups used for the distribution of the solutions

should not be utilized again but should be burnt or deeply buried as soon as they have been collected

Large-scale use of sodium fluoroacetate has been made by some workers with success and as has been shown by Macchiavello [119] and Macchiavello et al [1 2] the community wide distribution of this poison in combination with DDT application is an effective means of cutting short plague out breaks Nevertheless, in the opinion of most workers 1080 should not be utilized for general anti rat campaigns but should be employed only if possibilities for the primary or secondary poisoning of domestic animals as well as danger for human beings, can be fully excluded Certainly however one should not hesitate to utilize this most effective poison in locations where it is safe to do so for instance, during weekends in factories, godowns, schools and offices, or in other buildings which are not frequented at the time In emergencies, possibilities for the temporary evacuation and locking up of the premises to be dealt with ought to be given consideration

It deserves great attention that, as shown by the work of Hughes [90 91] quite satisfactory results may be obtained when using 1080 in place of fumigation procedures to free ships from rats As this worker summarized in 1950 [91] the ratio of rats killed by 1080 to that of rats estimated to be aboard was 85 5% in the case of the 96 vessels baited in 1946 and 1947, and 91 8% in the case of 283 vessels baited afterwards The value of this pro cedure which could be applied easily in the case of vessels of even smaller size plying on inland waterways as well as in seaports, is certainly considerable

So far no specific antidote for treating 1080 poisoning in man is known The symptomatic treatment to be used in such cases is outlined by Hughes [90] thus

> Ten-eighty is absorbed readily by the gastrointestinal tract and must therefore be removed immediately if harmful effects are to be prevented The patient should be made to vomit at once by sticking a finger in the throat or [the stomach should be voided] by other means Give a dose of magnesium sulfate (Epsom salt) or other cathartic as a purge
>
> In the event of nervous system excitation the careful use of barbiturates of medium duration of action, such as sodium amytal intravenously if necessary is suggested Other than complete rest and adequate sedation little can be done to prevent progression of cardiac symptoms Should ventricular fibrillation occur intracardiac injection of 5 cc of 1 percent solution of procaine hydrochloride might be attempted to restore an organized heartbeat Although symptoms of 1080 intoxication will usually subside within 1 day the patient should be kept quiet for a period of 3 days if there is any sign of action on the heart

It is interesting to note that as shown by Tourtelotte & Coon [216] dogs poisoned orally with approximately $2 \times LD_{50}$ of 1080 were invariably saved when barbiturate treatment was started half an hour or 3 hours after poisoning 80% of dogs which had received $4 \times LD_{50}$ of 1080 were saved when such treatment was started half an hour after poisoning only 17%

of those poisoned with the same dose were saved when the treatment was commenced after 3 hours In the case of dogs poisoned with $6 \times LD_{50}$ of 1080 barbiturate treatment was ineffective even if started half an hour after poisoning Sodium acetate and ethanol had some antidotal effect when given to dogs immediately after they had been poisoned with 1080 but these agents were of no value as adjuncts to barbiturate treatment when given half an hour after poisoning

Anti coagulants

The mode of action of the anti coagulants used for the purposes of rodent control is fundamentally different from that of the above described rodenticides The efficacy of the latter depends upon their administration in single doses adequate to kill the animals Single doses of the anti coagulants unless excessively large are incapable of exerting a lethal action However if repeatedly ingested in smaller or even quite small doses the anti coagulants because they inhibit the formation of prothrombin are apt eventually to prove fatal to the poisoned animals by producing haemorrhages in the tissues of the body and occasionally also external bleeding It is clear that this peculiar residual action of the anti coagulants can take place only if their admixture with the bait material is not recognized by the rodents because otherwise the animals would cease to consume the poison baits for sufficiently long periods

O Connor[15] who seems to have been the first worker to use anti coagulants for rat extermination as well as Diagne et al [40] stated to have obtained satisfactory results with dicoumarol (3 3 methylene bis 4-hydroxycoumarin) but as shown by Armour & Barnett[5] the consumption of this compound is apt to produce acquired bait refusal (bait shyness) in the rats However general agreement has been reached that 3 (α acetonyl benzyl)-4 hydroxycoumarin recommended as a potential rodenticide by Link and his co workers (see Scheel et al [187]) is free from this drawback and may be used therefore for the gradual eradication of rodent populations

This anti coagulant afterwards called warfarin is a stable colourless crystalline solid free from odour and taste not only for man but apparently also for the rodents It is available in a concentration of 0 5% in corn starch powder and may thus be easily admixed with dry bait materials It can be used in solution as well but, as stated by Upholt [18] ordinarily is not recommended for use in this form since one of its attractive features is the fact that it can be left in permanent bait stations with only infrequent checking Dry baits are obviously desirable from this standpoint "

The concentrations at which warfarin ought to be used vary according to the species of rodent to be dealt with As shown by Hayes & Gaines [81] and accepted in recent recommendations for the use of this anti coagulant (*US Public Health Reports* 1952 [174]) generally speaking a concentration of 0 05 mg/g of bait material (0 005%) is effective for the control of Norway

rats, but under certain conditions these rodents may be controlled a little more rapidly, though no more surely by use of bait containing 0 100 mg of warfarin per gram of bait '

Mice, though showing more individual variation, generally react in the same way as *R norvegicus* but a higher warfarin concentration (0 25 mg/g of bait material or 0 025%) is necessary for the dependable control of *R rattus* Doty [48] concluded from cage tests that *Rattus rattus alexandrinus* were comparatively most resistant to warfarin used in a concentration of 1/4,000 because they succumbed after having consumed 43 4% of their body weight of poisoned rolled oats as against 39 1% in the case of *R r rattus* and 23% in the case of the Norway rats

As established by field tests made by Gross et al [74] baiting with rolled oats to which warfarin had been added in a concentration of 0 925% by weight though satisfactory for the control of the common rats (particularly *R r alexandrinus* and *R norvegicus*) and also for *Mus musculus* did not reduce the incidence of *R hawaiensis*

Though, in order to be effective, warfarin containing baits must be ingested by the rodents several times, consumption need not necessarily take place on consecutive days Crabtree,[31] who experimented with albino rats noted in this connexion that results remained satisfactory if the poison baits were offered on 4 5 occasions at intervals of 1 4 days However, Link & Mohr [114] postulated in a recent summary that the intervals between warfarin feedings should not be longer than 48 hours

Laboratory investigations as well as field observations have shown that, *if warfarin is continually offered to susceptible rodents the mortality* becomes considerable after 4 5 days and most of the animals succumb within a week practically all within two weeks This is well illustrated by the experiences of Doty [48] who found that in cage tests 78 7% of the warfarin fed rats (*R norvegicus* and *R rattus*) were dead seven days after the first *ingestion of the poison and 95 4% after nine days* the few remaining animals surviving for 10 or 11 days Since in his opinion in actual practice four days had to be allowed for the average rat in the field to find a bait station and to start consuming the poison, Doty concluded that *to kill* 80 per cent would require a total of 11 days 95 96 per cent would require 13 days and positive 100 per cent kill would require 16 days , provided that no rats immigrated He proposed therefore that warfarin treated oats should be exposed in the field stations for at least 17 days or longer if there was active migration *Hence though the use of warfarin* obviated the necessity of prebaiting with unpoisoned baits, the period of its application was considerably longer than that required to prebait and then to expose the ordinary rat poisons—operations lasting a total of 9 10 days

Though warfarin may be admixed with all sorts of foodstuffs cereals *form the most suitable bait* bases for this poison because they facilitate a long exposure of the baits and reduce the danger of their consumption by cats

and dogs. As noted above, Doty[18] and also Gross and his collaborators[71] used rolled oats to prepare warfarin baits, while workers in the USA considered yellow corn meal of a cooking grade as the bait of choice. Since, as stated above, warfarin is used in rather low concentrations, thorough mixing of the poison with the bait material is essential. If it is proposed to leave the baits exposed instead of replacing them at frequent intervals, the addition of 0.25%–0.4% of *p*-nitrophenol is advisable so as to prevent the formation of moulds.

In order to derive maximal benefit from the residual action of warfarin many workers recommended not distributing the poison baits in the usual manner but exposing the material in bait boxes. This procedure, which will be dealt with in a general manner later (see page 560), has the double advantage of making the poisoned material permanently available to the rodents and preventing its consumption by larger domestic animals or by children.

Like ANTU, the anti-coagulants may be used as tracking powders laid down in sufficiently large quantities at strategic points such as the entrance of rat burrows and rat runs. One proprietary brand in particular, containing a coumarin derivative (Reiff & Wiesmann[1 6]) in a concentration of 1%, has given excellent results when distributed in this manner.

As shown by these experiments as well as by those made when using anti-coagulants in bait form, advantage may be taken of these substances for the purposes of "initial" rodent control. However, it must be realized that if used in this manner the anti-coagulants not only act considerably more slowly than the usual rodent poisons but are also much more expensive to use. Doty[18] noted in the latter connexion that "a study of comparative cost of materials for poisoning rats under our field conditions showed warfarin to be 50.7 per cent higher than thallium sulphate and 78.3 per cent higher than zinc phosphide".

While it may be necessary therefore to utilize other rodenticides for initial anti-rodent campaigns, the anti-coagulants, because they can be employed continually, are of eminent value in keeping the premises in which other procedures had been used to decimate the rodent populations permanently free from these animals. Indeed, on account of their residual action warfarin and similar compounds offer "a sort of chemical ratproofing in places where a conventional ratproofing is impractical for economic or other reasons".[3]

It also deserves great attention that, as pointed out by Hayes & Gaines,[81] warfarin appears to be most suitable for keeping ships permanently free from rats.

As generally held, the danger of direct poisoning of domestic animals through ingestion of bait materials containing anti-coagulants is not considerable and, as noted above, this danger may be practically eliminated by exhibiting these materials in bait boxes. However, instances of secondary

poisoning in cats and dogs which consumed large numbers of warfarin poisoned rodents have been observed To counteract this danger as far as possible, prompt collection of the poisoned rodents must be insisted upon, particularly during the period immediately following the commencement of 'initial' anti rodent campaigns

As far as is known, the distribution of bait materials containing warfarin or *similar compounds has never led to untoward incidents in man*

It is curious to note in this connexion the case of a young man who, in order to commit suicide, ingested during a period of 6 days a total of 4 ounces (113 g) of a warfarin preparation containing 567 mg of the anti coagulant He showed on admission four days after consumption of the last dose a petechial rash and a brownish red discharge from the nostrils There was laboratory evidence of hypoprothrombinaemia Red blood corpuscles were found in the urine which afterwards showed temporarily gross admixture of blood The stools were of normal colour but one bowel movement was followed by voiding of blood However, administration of vitamin K and blood transfusions as recommended for the treatment of poisoning with dicoumarol *and related substances effected recovery within less than* a week (Holmes & Love [87])

Other rodenticides

In addition to the above mentioned widely known rodenticides the following substances found to be poisonous for rodents deserve *attention* even though some of them have not yet been utilized for actual control work

1 2 chloro 4 dimethylamino 6 methylpyrimidine Castrix was devel oped during the second World War by German scientists so as to replace rodenticides which had become unavailable Du Bois et al [49] reported in 1948 that this compound produced in all animal species studied symp toms typical of central nervous system stimulants particularly convulsions, which began to appear 15 45 minutes after either oral or intraperitoneal administration

Castrix was found to be more toxic to laboratory rats than either ANTU or 1080, the lethal dose (LD_{50}) being $1\ 25 \pm 0\ 10$ mg per kg body weight *when given orally, and* $1\ 00 \pm 0\ 06$ when administered intraperitoneally The LD_{50} for mice given the poison intraperitoneally was $0\ 42 \pm 0\ 05$ mg per kg, the corresponding figure for dogs was about 0 50 Rats first given *sublethal doses of Castrix developed* no tolerance to the poison They appeared to be more susceptible to it in summer than in winter

It is important to note that sodium pentobarbital was found to be an effective antidote against at *least 10* LD_{50} *of this poison in* rats and dogs even if administered after convulsions had appeared Kalmbach [101] main tained therefore that 'since it can be antidoted it may prove to be of

advantage to use Castrix as a raticide rather than sodium fluoroacetate (1080) in many instances' So far however this advice does not seem to have been followed

2 Guillaume [7] obtained satisfactory results in rat control with gluco chloral admixed with solid bait materials, particularly grain, or with bouillon in a proportion of 20% The use of this substance did not seem to lead to instances of secondary poisoning in domestic animals

To judge from limited experiences made by Tara [15] glucochloral was effective against commensal mice even if used in a concentration of 1/1 000

3 As established by Karel [103] the phenylhydrazine derivative of fluoroacetic acid Fanyline was readily accepted by albino rats and mice when offered in corn meal in concentrations of 1% or 2%

The oral median lethal doses of this compound, expressed in mg per kg body weight may thus be compared with the corresponding values obtained with 1080

Species	*Fanyline*	*1080*
Albino rat	9 1 ± 2 7	2 5 7
Albino mouse	44 9 ± 2 9	8 — 10 (commensal mouse)
Cat	greater than 0 25 less than 0 5	0 35 0 50
Dog	0 1-0 25	0 07 0 20

As will be noted Fanyline while less poisonous for albino rats and markedly less poisonous for mice than 1080 was but slightly less dangerous for dogs than the latter The toxicity of Fanyline for cats was quite similar to that of sodium fluoroacetate

Karel [103] *expressed the opinion that the lethal action of Fanyline* was due to the fluoroacetate moiety of the molecule He considered as an advantage the fact that in contrast to 1080 Fanyline was but slightly soluble in water

4 Studying the properties of the nitro dyes (colorants nitres) and possibilities for their use Pastac [1 9] found that certain of these substances such as Martius yellow (2 4 dinitro α naphthol) and Victoria yellow (2 4 dinitro *o* cresol) because physiologically active and at the same time colourless and tasteless appeared to be suitable for the eradication of rats or other harmful animals He ascribed the toxic action of these substances to a catalytic activation of the intracellular respiration which because it rendered the absorption of a sufficient amount of oxygen impossible led to death from asphyxia

These nitro dyes the lethal dose of which was 0 01 g per kg of body weight could be admixed with all usual solid bait bases

During the 1946 anti rat campaign at Marseilles Bestieu [14] actually used dinitro *o*-cresol in premises where a consumption of this poison by domestic animals could be prevented Baits were prepared by impregnating

small pieces of bread, which had been previously coloured with methylene blue, with a 2% solution of the poison in a proportion of 500 ml per kg of bait The poisoned rats were found to succumb within two hours their carcasses showing evidence of desiccation

5 As stated by Kalmbach,[101] some of the organic phosphates designed originally as insecticides such as hexamethyltetraphosphate, tetraethyl pyrophosphate, and parathion ('Thiophos') were found to be highly toxic to laboratory rats Kalmbach was careful to state, however, that these highly toxic substances were particularly dangerous because they were readily absorbable through the unbroken skin

Kalmbach added that attention was paid to the possibility of using thiosemicarbazide which had given satisfactory results with laboratory rats against orchard mice

It may be conveniently added that, besides poisons, inoffensive substances such as plaster of Paris have been recommended for the purposes of rodent eradication with the idea of producing in this way, a mechanical obstruction of the gastro intestinal tract of the animals Cors[30] used a mixture consisting of 6 parts of plaster of Paris with 2 parts of wheat flour and 1 part of sugar for this purpose

Mixtures containing unslaked lime instead of inoffensive substances have been recommended as well so as to produce burns of the stomach mucosa when the lime becomes slaked through the action of the gastric juice Using this method in grain stores and in fields Ivanow & Djarewa[97] were very successful in the case of *M musculus*, but obtained only 60% 70% success with *R norvegicus*

Rules for conducting poisoning campaigns

The *methods of conducting poisoning* campaigns against the commensal rodents particularly against the rats which are most amenable to this mode of control, have been greatly improved during recent years not only owing to the introduction of more efficacious rodenticides but also on account of much progress in the knowledge of the biology habits and reactions of these animals The procedures now adopted for the conduct of the campaigns may be set forth thus

General principles

While in the past, poisoning of commensal rodents was often done in a piecemeal manner by dealing only with single houses or small groups of houses it is now realized that, in order to prevent a rapid re infestation of the treated premises, the poisoning campaigns must be conducted on a large scale Whenever possible the work should be carried out at one and the same time on a community wide scale failing that it ought to embrace whole precincts or at least blocks of buildings

It has likewise been realized that poisoning of the commensal rodents must be done in an intensive as well as in an extensive manner by dealing with all foci of infestation within the areas concerned. It is essential therefore to pay attention not only to all infested buildings including — besides residences — outhouses and godowns, but also to other rodent locations such as garbage dumps and sewers. Recent experiences in Great Britain have proved the great importance of such a "vertical" system of rodent control including the exposure of poison baits in the sewers as well as in the buildings above them (Barnett[7]).

Initial surveys

Though as summarized by writers such as Dice[41] and Emlen et al.,[54] various methods have been recommended for determining the numbers of small mammals such as the commensal rodents, only some of these procedures are of practical value for assessing the degree of infestation before poisoning campaigns. These are: (*a*) questioning the people regarding the presence and frequency of the rodents; (*b*) estimating, by means of counting, the number of rodent burrows or tracks present or the number of rodents seen; (*c*) systematically searching for droppings and other signs of infestation; and (*d*) determining the consumption of unpoisoned baits offered to the animals.

Unless the number of rats or mice is small, the people are, as a rule, able (though not invariably willing) to tell whether or not these animals are present in their houses, outhouses or godowns. Usually, however, not much credence can be given to statements made by the people in regard to the degree of rodent infestations.

Counting the rodent burrows gives, as a rule, no reliable results because in buildings their number is seldom related to the density of the rodents (Chitty[6]). Likewise an inspection of the tracks made by the animals in dust, mud or snow or in deposits of plaster of Paris or similar materials laid down for this purpose (fig. 36 and 37), while showing the presence of rodents, will give little indication as to their number (Chitty,[6] Morgan[11]).

Gunderson[8] maintained that on ships in particular it was possible to determine the degree of rat infestation by counting the number of animals seen. However, Chitty[6] stated that, owing to the nocturnal habits of the rats, no representative fraction of them was likely to be seen in daytime except when methods such as harbourage destruction or bolting the animals with gas or ferrets were used. Emlen et al.[54] found the counting method of some usefulness in places such as public markets where the presence of long corridors and continuous artificial illumination created unusually good facilities for observation. However, in the opinion of these workers "indices so obtained should be based on repeated counts combined with a careful analysis of factors in the local situation. Rarely can they be expected to provide more than a crude index."

The kinds and amounts of droppings and other signs useful for an estimation of the degree of rat infestation are shown by the following tabulation culled from *Rat borne disease prevention and control* [17]

Signs observed	Degree of infestation (number of rats present) Light (1 20)	Medium (21 50)	Heavy (over 50)
Fresh droppings	None or few groups generally all of same size	Some always observable in two to six or eight areas usually two distinct sizes	Many usually of several sizes small to large and in at least six locations
Active runs and smears	None or few and relatively indistinct	Several distinct one or more indicating heavy travel	Many more than one heavily travelled run
Fresh gnawings	None to few nightly	Usually several instances nightly	Many instances nightly

On board ship in particular, it is often possible, by paying close attention to these signs of rat infestation, particularly to the droppings, to arrive at a fairly accurate estimation not merely of the degree of infestation but of the number of rats present However, great experience is necessary to make such estimations reliable Moreover as recently pointed out by Morgan,[15] a number of extrinsic factors may render a correct interpretation of the results difficult or even impossible Morgan upheld in particular that no accurate estimation of the rat population can be made with the cargo *in situ*

More reliable estimations of the number of rats present in buildings or other locations on land as well as on ships can be obtained by offering an ample weighed amount of plain bait material to the animals determining the consumption of this food by weighings repeated at intervals of 24 hours, and dividing the amounts thus found by the approximate quantity of food eaten by one rat per day (25 30 g according to Chitty & Shorten [7] in the case of *R norvegicus*) However, such determinations show merely the probable minimum population of the rodents because young animals are apt to eat less than the adults and also because the latter do not necessarily consume their full quota (Morgan [15]) It has to be noted, however, that the rodents if offered the unpoisoned bait material for several days become accustomed to partaking of it freely

Public health propaganda

It is essential for the proper conduct of rodent poisoning campaigns to obtain the co operation of the public by the use of all available methods of public health propaganda In particular, it is of great importance to enlist the help of the people in preventing access of children and of domestic animals to the poison baits and the consumption of poisoned rodents by the latter At the same time the people must be urged to promote bait consumption by the rodents through making other food materials as far

The kinds and amounts of droppings and other signs useful for an estimation of the degree of rat infestation are shown by the following tabulation culled from *Rat borne disease prevention and control* [17]

Signs observed	*Degree of infestation (number of rats present)* Light (1 20)	Medium (21 50)	Heavy (over 50)
Fresh droppings	None or few groups generally all of same size	Some always observable in two to six or eight areas usually two distinct sizes	Many, usually of several sizes small to large and in at least six locations
Active runs and smears	None or few and relatively indistinct	Several distinct one or more indicating heavy travel	Many, more than one heavily travelled run
Fresh gnawings	None to few nightly	Usually several instances nightly	Many instances nightly

On board ship in particular it is often possible by paying close attention to these signs of rat infestation, particularly to the droppings, to arrive at a fairly accurate estimation not merely of the degree of infestation but of the number of rats present However, great experience is necessary to make such estimations reliable Moreover, as recently pointed out by Morgan,[14] a number of extrinsic factors may render a correct interpretation of the results difficult or even impossible Morgan upheld in particular that no accurate estimation of the rat population can be made with the cargo *in situ* '

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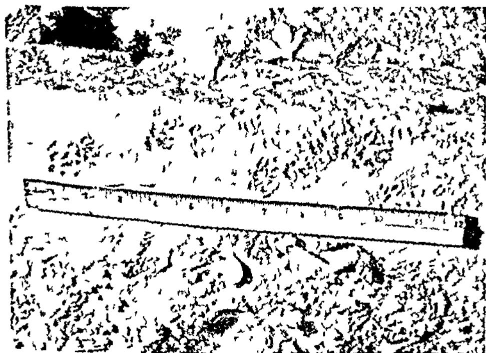
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FIG 36 RAT TRACKS AND TAIL MARKS IN THE MAKING



FIG 37 RAT TRACKS IN DUST



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The various ingredients used for the preparation of poison baits particularly the rodenticides themselves must be accurately weighed Thorough mixing is essential so as to distribute the poisons evenly

Workers in the USA often recommend the use of several (2 4) different types of bait base during one and the same poisoning campaign However elsewhere fully satisfactory results have been obtained with the use of only one type of bait base It is however indispensable to employ different bait bases in follow up treatments or for quickly repeated campaigns, because rats which have not succumbed after ingestion of a poison bait may refuse to partake of the same bait material even if a different poison has been admixed with it In fact as stated by Barnett[9] on the whole shyness develops more readily to the bait base (e g flour) than to the poison although physiologically it is the poison that is responsible for it Consequently the routine procedure used in Great Britain for dealing with surface rat infestations was to distribute initially 2 5% zinc phosphide in sausage rusk to bait three weeks later with sugar meal containing 15% arsenious oxide and if a third treatment was necessary to use 10% red squill admixed with bread mash[7]

As maintained by Barnett[7]

> mice do not develop bait base shyness although they seem to show a general shyness of food after sublethal doses of poison There is therefore no need to change the bait base for the second treatment The general shyness can be overcome by prebaiting 4 days will usually be sufficient but sometimes up to 10 days is needed

While as noted before it is preferable to use 1080 in the form of watery solutions as a rule the other rodenticides are admixed with different solid bait bases Materials such as rolled oats or other sorts of grain flour or meal may be distributed loose or wrapped up in waxed paper Water or some kind of oil may be used to prepare a dough or paste with the bait material to which the poison is added and the bait mass is then distributed in the form of balls or small cakes which may be dipped in hot oil to make them firmer and more attractive to the rodents As already described diced ½ inch (1 3 cm) cubes of vegetables or fruit may be coated with the rodenticides To distribute the poisoned bait material in loose form is advantageous because the rodents are not apt to carry it away as they often do in the case of wrapped up or piece baits Moreover such loose bait material if distributed in small quantities (5 g corresponding to one level teaspoonful) is little apt to attract the attention of cats and dogs On the other hand wrapped up or piece baits which should also be used in 5 g doses can easily be inserted into sheltered sites such as rat burrows In actual practice it may be well therefore to prepare both loose and wrapped up or piece baits so as to be able to deal adequately with the different sites to be baited

As mentioned before p nitrophenol may be used to prevent mould formation in warfarin containing grain Other methods recommended in

as possible, inaccessible to them While generally speaking the people should also fight the commensal rodents by reducing possibilities of harbourage, such clean up operations should not be undertaken immediately before poisoning campaigns so as not to disturb the animals

Choice of poisons

The choice of rodenticides to be used during individual poisoning campaigns depends upon several variable factors, particularly the species of commensal rodents present, the nature of the sites to be dealt with, the attitude of the people and the available funds and facilities Of great importance are also the reasons for which the campaigns are undertaken Thus, in emergencies created by the presence or threat of plague outbreaks rapidly acting poisons should be given preference, while in plague free times or localities the use of anti coagulants ought to be considered

Since commensal rodents which have consumed sublethal doses of a rodenticide may become poison shy, refusing to consume further baits containing the same poison it is not advisable, in general, to use several rodenticides during the same campaign because then no suitable poison might be available for follow up treatment or campaigns to be repeated at short intervals However, it may be advantageous to use in addition to the general distribution of less dangerous poisons 1080 in sites inaccessible to children or domestic animals

Choice of bait bases and bait preparation

The appropriateness of bait bases depends not only upon their compatibility with the poisons chosen but also upon their acceptability for the rodents to be dealt with It is of utmost importance to realize in this connexion that bait materials found fully acceptable in one locality may prove quite unsatisfactory in another Hence before definitely choosing the bait base or bases to be used in a poisoning campaign one must never omit to ascertain their acceptability by means of test baiting i e, by distributing small quantities of the unpoisoned bait materials in the form in which it is proposed to use them, and then observing how well these materials are consumed by the rodents

It is often maintained that different bait bases should be chosen to cater for the different food preferences of (*a*) the Norway rats more inclined to be meat and fish eaters and (*b*) *R rattus* said to prefer vegetables and fruit Actually however simple bait bases such as grain or sausage rusk (a biscuit meal used as filler for sausages) are often fully acceptable to both rat species and to the mice as well To use such plain bait bases is desirable for the sake of economy and expediency of bait preparation Moreover plain baits are less attractive to cats and dogs than those containing meat or fish

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the handbook on rat borne disease [17] for preventing spoilage of poison baits are (*a*) to use vegetable oils instead of water or milk to prepare solid baits and (*b*) to coat the baits with a tasteless material such as glycerol However the use of such *preservatives is not indicated in the case of highly toxic* poison baits which must be promptly collected and discarded, if not rapidly consumed

The precautions to be taken when handling poisons absorbable by or irritating to, the skin, for the purpose of bait preparation, have been referred to already when dealing with the individual rodenticides

Prebaiting

As summarized by Barnett [9] in a valuable report on the principles of rodent control on account of their reaction to new objects rats may at first completely avoid partaking of an unfamiliar food offered to them or may even avoid food to which they are accustomed, if it is offered at a new place After this initial stage the rats tend to go through a phase of tentative nibbling It follows that if poisoned bait materials are offered *to the rats straight away, the latter may consume these baits only in sublethal* doses, if at all and may, therefore, become poison shy and/or shy to the bait base used To overcome this impasse, the procedure recommended is to distribute, first unpoisoned bait materials and to use poison baits prepared with exactly the same foodstuffs only after the latter have been available to the rats for some (usually four) days, so as to accustom them to the consumption of the given food at the given places

This method of prebaiting seems to *have been used first by* Pemberton (1925) [162] and by Doty (1938) [46] in Hawaii, but—as stated by Martorana [139]—early mention of it was also made by Schauder & Gotzer (1930) [186]

Prebaiting may be done in two different ways, either by offering to the rats a surplus of bait material or by exhibiting amounts smaller than the quantity the animals could consume (token baiting) A kind of combination of these two methods was recommended by Storer [21] thus

> A given weight of prebait—say 2 ounces [60 g] which may be measured by bulk with a suitable spoon—is put out at each station On succeeding days if much is eaten the station is refilled doubling the amount each time (4 8, 16 ounces etc [120 240 480 g]) and this practice is continued until the amount taken levels off when a maximum number of rats will be eating at the station

The method proposed by Storer, though time consuming is advantageous if one wishes to use prebaiting procedures not only to overcome the new object reactions of the rats but also *for purposes* of census taking because it gives more reliable results in the latter respect than short term surplus baiting

Though as shown by Barnett et al,[10] one may arrive at an estimate of the number of rats by comparing the consumption rate of token baits at different feeding points results obtained in this way did not always

tally with those obtained through surplus baiting However token baiting is fully satisfactory if one wishes merely to accustom the rats to the bait material and feeding places Indeed, in the opinion of Chitty & Shorten [27] token baiting was more satisfactory for this purpose than surplus baiting Certainly it is less expensive than the latter procedure, because, as stated in the British instructions [7] it is sufficient when dealing with surface infestation to prebait with 1 2, or 4 ounces (30 60 or 120 g) on each of four consecutive days and to use prebaits of 4 ounces (120 g) daily for two days in the case of sewers

While prebaiting has become a standard practice in Great Britain and is also amply used elsewhere particularly in Hawaii some workers in the USA do not consider this procedure indispensable for successful rat poisoning According to the handbook on rat borne diseases [217] prebaiting is recommended in difficult poisoning cases, but not in a routine poisoning program

Since the commensal mice display little or no new object reactions (Southern quoted by Barnett 1948 [9]) there is certainly no need to use prebaiting procedures in their case Prebaiting is also unnecessary before poisoning campaigns with warfarin or similar compounds the action of which depends upon the repeated ingestion of bait materials containing these substances

Distribution of poison baits

In order to poison commensal rodents baits may be laid (*a*) in holes (*b*) on the surface and (*c*) in containers

Hole baiting is a satisfactory method the more so because one can easily ascertain the results of poisoning by blocking all the openings of the burrows in question after the rodenticides have been inserted any rodents which survive will have to re open one or some of the holes

Unfortunately however the scale on which hole baiting can be done is apt to be limited the rats often living in other locations instead of in burrows

Surface baiting while the least safe of the three above mentioned procedures is quite effective if care is taken to deposit the baits so that the rats are intercepted between their nesting sites and their usual sources of food (Barnett 1947 [8]) Therefore whenever possible the poison baits should be laid near the mouths of burrows or near the rat runs but not directly on the latter because as the handbook on rat borne disease [17] justly says rats prefer to investigate rather than stumble over baits Generally speaking the baits should be deposited in dark secluded corners or sheltered locations so that as stated in the above mentioned handbook [17] an untrained individual should not see many of the baits after a trained man has placed them properly

(375 mm) long and with an internal diameter of 3 inches (75 mm), which have been blocked at one end As stated by Barnett,[7] poultry can reach inside these pipes, but some protection may be achieved by placing them with the open end 3 inches (75 mm) from an object such as a wall or a brick

Special techniques have to be used for the baiting of garbage and refuse dumps and of sewers

Since as stated by Schuler,[188] the distribution of red squill baits on garbage and refuse dumps gave unsatisfactory results, and calcium cyanide dust could not be used there on account of the presence of numerous openings highly toxic poisons had to be employed Pending their exposure it was necessary to have guards on constant duty or to fence the dumps safely It was preferable, however, to use bait boxes for prebaiting and baiting As an alternative, baits which became non toxic in a few days could be distributed Schuler[188] recommended for this purpose the addition of two parts of zinc phosphide and one part of magnesium carbonate to 16 parts of rolled oats and 48 parts of ground horse meat or fresh fish He maintained that, if placed under cover on rat-trails or in burrows these baits became decomposed to a non toxic residue after several showers of rain

For poisoning work in sewers, Barnett[7] recommended special bait trays and bait depositors which were lowered through the manholes Poisoning was first to be done, after two days prebaiting, with zinc phosphide, and four weeks later with arsenious oxide in a different bait base Whenever necessary single maintenance treatments were to be carried out at six monthly intervals It was permissible to use the previously employed poisons and bait bases for such repeat treatment because there was no evidence to show that bait shyness persisted in wild rat populations for as long as six months (Barnett, 1947[8])

Whatever modification of rodent poisoning methods is used, one must always realize that, except in the case of the anti coagulants, the success of the poisoning campaigns depends upon a large initial kill Hence it is of utmost importance to avoid underbaiting by offering an amount of poison bait more than sufficient for the rodents estimated to be present As stated in this connexion in *Rat borne disease prevention and control*[17]

> A major fault in unsuccessful poisoning programs is the use of too few baits Many times an individual will report great success because every bait was taken This may however simply mean that there were many more rats than baits One hundred to two hundred baits of each type is not considered excessive for a heavily infested business establishment Since there are about 80 to 90 individual baits per pound this would not be expensive treatment "

Similarly bait boxes must be kept constantly supplied with an amount of poison bait sufficient to feed all rats coming to them It should be noted however, that the amount of poison bait consumed may be considerably lower than that of prebait eaten at the same feeding stations Storer[1]

maintained in this connexion that a guess as to the number killed can be made on the basis of 1/3 ounce (10 g) per rat

For the sake of safety it is best to distribute the poison baits as late as possible in the afternoon and to collect those which have not been consumed as well as the dead rodents as early as is feasible on the next morning This procedure is also advantageous in so far as it permits of preparing the baits on the day on which they are laid

To facilitate the collection of unconsumed baits, the places in which baits are deposited should be marked with chalk or by affixing small labels bearing the inscription Poison—do not touch During bait distribution a final warning must be given to the people to safeguard children and domestic animals

If it is quite safe to do so, poison baits may be left in position for one or two more nights Even then however, the baited places must be visited every morning in order to collect the dead rodents

Disposal of unconsumed baits and poisoned rodents

As has been stated already unconsumed baits as well as the poisoned rodents must be destroyed by incineration or must be buried to a depth of at least 2 feet (60 cm) No other method of disposal is safe

A serious nuisance may be created during poisoning campaigns by rodents dying and decomposing in places which cannot be reached without major damage to the structures in question e g within double walls For such cases the blowing in of deodorants such as powdered activated charcoal chloride of lime or solutions of formaldehyde lead acetate or lead nitrate has been recommended (Storer [1]) Should this be impossible deodorants such as pine oil or wintergreen oil should be sprayed as near as possible to the source of the odour A mixture of naphtha flakes in white gasoline is particularly effective for this purpose but cannot be used if there is a fire hazard [1]

Assessment of results of campaigns

It is generally agreed that the number of carcasses recovered after the distribution of poison baits does not indicate the success obtained because even if most of the rats or mice have been killed not many or even only a few of them may be found dead Stress has to be laid therefore upon ascertaining the number of survivors rather than upon counting or otherwise assessing the number of casualties

Some workers used trapping procedures to assess the number of surviving animals but the reliability of this method was doubted by other observers Two methods generally considered as valuable for appreciation of the results of poisoning campaigns are the following

(1) Close inspection by experienced observers for the signs of rodent infestation mentioned earlier If owing to a decimation of the rodents

signs of infestation are insignificant, it may be helpful to sprinkle plaster of Paris or other dusts near the shelters or on the runs of the animals and to study the tracks made by them

(2) Post baiting, done by distributing unpoisoned bait materials different from those used during the campaigns and watching the consumption rate of these foodstuffs It is generally agreed that post baiting should not be commenced immediately after poisoning but only after an interval of one or preferably two weeks As maintained by Chitty & Shorten, [7] a surplus of material should be used for post baiting

If the use of this method indicates the necessity for further poisoning work, it is legitimate to carry this out with the aid of the bait base utilized for the purpose of post baiting Accordingly, the British instructions[7] recommended for the fourth week of poisoning campaigns

Lay test baits of sugar meal at each point baited in week 1 Where bait is taken by 3rd day continue to bait at these points poison on 5th day with 15% arsenious oxide in sugar meal

Virus

The various brands of "virus used for the purposes of rodent control are actually bacterial strains, mostly those belonging to the *Salmonella enteritidis* group (see Leslie [111]) distributed with the idea of producing fatal infections not only in the rodents which directly consume these materials but also in those which come in contact with, or devour the carcasses of the original victims

Opinions regarding the efficacy of this method and the advisability of using it are sharply divided It was warmly recommended by French workers in particular, more recently by Petit (1936, 1943) [163, 164] and Auvray (1942) [6] for instance, and is still widely used in France

With very few exceptions recent observers in other countries consider the method of using virus not only as ineffective but also as rather dangerous for human beings In fact, numerous cases of human gastro enteritis due to the distribution of virus preparations for the purposes of rat control are on record Leslie,[111] who summarized the evidence available in this respect up to 1942 added that "there are also, reasonable grounds for believing that these bacterial types may be pathogenic for a number of domestic animals including some poultry'

The unfavourable opinions held by most plague workers in regard to the above described method have been endorsed by a resolution passed by the WHO Expert Committee on Plague in 1952,[237] which stated that "the experts were definitely against the use of virus preparations as rodenticides

Fumigants

Lethal vapours or gases produced from the following substances have been found useful for killing commensal rodents and in part also their ectoparasites

Carbon disulfide

Carbon disulfide (CS_2) is a colourless liquid with a boiling point of 46 3°C It is readily volatilized at ordinary room temperature, the vapour being 2 63 times as heavy as air The vapour which has an unpleasant odour is extremely inflammable and apt to be explosive when mixed with air for instance when coming in contact with hot surfaces such as those of steam pipes (Frear[56])

Though mainly important as an insecticide carbon disulfide has been used on a fairly large scale for the destruction of wild rodents in California South Africa and south east Russia (see section on the control of wild rodents page 582) and of free living commensal rats in Hawaii[80] The usual technique was to introduce absorbent materials soaked with carbon disulfide into the burrows and then to plug the burrows

As will be gathered carbon disulfide has to be handled with extreme care It is far too dangerous to be used in buildings

Carbon dioxide

Carbon dioxide (CO_2) in solid form (carbonic acid snow) which is widely employed as a refrigerant has occasionally been used to kill rats (Pieniazek & Christophers[165]) and mice (Barnett[9]) It does not seem to act as a poison but when evaporating and thus displacing the oxygen in the air merely suffocates the rodents Carbonic acid snow must be handled with spoons made of horn or plastic and not with the bare hands on which it may produce burns

Carbon monoxide

In the past ample use has been made of the mixture of carbon monoxide (CO) and CO generated with the aid of a complicated apparatus usually installed on a barge for the purposes of ship fumigation However little if any use is now being made of this method mainly on account of its inability to destroy the rat fleas

According to Kalmbach[10] in the USA some use has been made of carbon monoxide which may be obtained in crude form from the exhausts of motor-cars to deal with rodent infestations in outdoor storage installations As stated in the handbook on rat borne disease[17] rat burrows in particular may be treated in this way by attaching a flexible hose to the exhaust pipe of an automobile running the tube into the burrow and operating the motor on a rich gasoline mixture with the choke pulled out It is unlikely however that this procedure which on account of the

lethal action of the exhaust gases on man must be used with great precaution, exerts a reliable action on the rat fleas, because, as established by De Raadt,[1 5] dry heat has to be applied in combination with carbon monoxide to kill these insects De Raadt found that in this way *Yenopsylla cheopis* were killed after *exposure to carbon monoxide at a temperature of 50°C for 45 minutes*

Chloropicrin

Chloropicrin or trichloronitromethane (CCl_3NO_2) is a slowly volatilizing colourless fluid, the vapours of which produce even at relatively low con centrations, intense irritation of the eyes—a property which renders chloropicrin useful as a forewarning agent in hydrocyanic acid fumigation

Besides being used as an insecticide, chloropicrin has occasionally been utilized for the purposes of rat destruction for instance by Lewis et al [11] who, during a plague outbreak at Dakar, poured the fluid on the floor of huts and then covered these for 24 hours with tarpaulins It should be noted in this connexion that, according to Sherrard,[198] the minimum lethal concentration of chloropicrin vapours for Norway rats was 1 ounce per 1 000 cubic feet at a temperature of 63°F (1 g per m^3 at 17 2°C) and with an exposure for 4 hours

Frear [56] stated that chloropicrin is superior to certain other fumigants in its complete freedom from fire and explosion hazards ability to penetrate bulk commodities, non reactivity with metals, fabrics and colors under *fumigating conditions, and has a pronounced odor and lachrymatory* effect, so that no ' warning gas need be added In the experience of Sherrard,[197] it also exerted no deleterious or toxic action on foodstuffs, which could be consumed without ill effect after exposure However the action of chloropicrin is slower than that of hydrocyanic acid, it is relatively toxic to living plants and seeds and its odour is apt to persist for days (Frear [56] Martorana [1 9])

Hydrocyanic acid

Hydrocyanic acid (hydrogen cyanide—HCN) in the pure state is a colourless liquid below its boiling point (26°C or 78 8°F) and is a colourless gas above this temperature It has a distinctive odour which, however, is not marked enough to warn inexperienced persons and causes no dis comfort even if the gas is present in lethal concentrations (Williams [31])

If used for purposes of fumigation, HCN—one of the most rapidly *fatal poisons known—is apt to cause death not only* when inhaled but also because it easily penetrates ordinary clothing and is then readily absorbed by the skin In the opinion of Williams [?34] workers provided with efficient gas masks might remain for half an hour in air containing 2 ounces of HCN per 1,000 cubic feet (2 g per m^3) *without experiencing* signs of poison ing, but should not stay longer than 5 minutes if the concentration of the gas amounts to 8 ounces per 1 000 cubic feet (8 g per m^3)

Generally speaking hydrocyanic acid gas possesses most marked penetrating powers but as aptly stated by Williams [231] penetration being merely one feature of diffusion it is not surprising to find that the gas also rapidly passes out of materials it has penetrated Hence unless excessively heavy HCN concentrations have been used and as long as the weather is dry a comparatively short airing will remove most of the gas from fumigated articles so that, for example, an hour s airing will render a mattress treated with the gas safe to sleep on However since water absorbs HCN and holds it specially when the weather is cold moist articles must be aired longer than dry ones

As a rule, the gas absorbed by water is given off so slowly as not to prove dangerous but this may not hold true if absorption of a comparatively large amount occurs on a cold day and warm weather follows quickly

In the concentrations generally used for fumigation in rooms HCN is not absorbed by foodstuffs in dangerous concentrations so that it is sufficient to air these articles for two or three hours before they are consumed However, food subjected to higher concentrations (10 20 ounces per 1 000 cubic feet or 10 20 g per m^3) as used in fumigation chambers, must be aired for 24 hours at least (Williams [231])

If applied in these higher concentrations HCN fumigation is injurious to delicate vegetables such as lettuce and probably also to bananas interfering with their ripening Further as pointed out by Williams [31] eggs do not as a rule hatch because their shell is penetrated by the gas Otherwise HCN fumigation is not injurious to commercial commodities even including fragrant articles such as tea and tobacco Its application is cheap the amount of 2 ounces of HCN per 1 000 cubic feet (2 g per m^3) as required for rat destruction costing according to Williams (1931) [31] 12½ cents

Hydrocyanic acid may be used for the purposes of rodent—as well as of insect—control in the following ways

(1) It may be generated on the premises to be treated (*a*) through the action of 50% sulfuric acid on sodium cyanide or (*b*) by adding sodium cyanide as well as sodium chlorate to 50% hydrochloric acid

(2) Rapid evaporation of the gas may be effected by (*a*) releasing liquid HCN which has been prefabricated and filled into heavy steel cylinders or (*b*) distributing porous inert materials such as fuller s earth paper or wood pulp discs in which liquid HCN has been absorbed in the premises to be dealt with

(3) Dusting with calcium cyanide from which HCN is released less rapidly under the action of the air moisture

Little use is at present made of the tedious generation methods mentioned in (1) The procedures mentioned in (2) are amply used for the purposes of ship fumigation and also to some extent for the disinfestation of ware

houses and of establishments such as mills Since, however, they can be applied efficiently and safely by specially trained workers only, they are not suitable for anti plague work in general The use of calcium cyanide, on the contrary, is of importance for the latter and will, therefore be dealt with in a detailed manner

Calcium cyanide

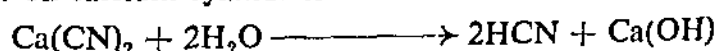
Calcium cyanide ($Ca(CN)_2$) is available in the form of a fine greyish white powder suitable for pumping operations and in different coarser granulations to be distributed or scattered in sites where a slower HCN evolution is desired One commercial brand has been supplied in the form of tablets together with a special pump which first grinds the tablets and then blows out the resulting fine powder

The calcium cyanide and HCN contents of the various brands available vary considerably as shown by the following findings made by Sokhey et al [05]

Brand	*Percentage of* $Ca(CN)_2$	*HCN*
A	42 30	23 31
B (tablets)	84 94	46 80
C	—	19 78

Measuring the yield and the rate of evolution of HCN from these three preparations, Sokhey et al [05] found that products A and B on coming in contact with the air, gave up their total HCN content, the maximum yield being reached in the case of B in 15 minutes and in the case of A in 30 minutes Preparation C yielded only about half its HCN content, and it took 90 minutes to reach even this low concentration It should be noted in this connexion, that in the experience of Metzger [133] calcium cyanide when spread in extremely thin layers yielded about three quarters of its HCN content in half a minute and practically all of it in one minute This gas evolution took place even if the air humidity was 25% or less

It is generally held that the chemical reaction produced by the action of moist air on calcium cyanide is as follows



$$Ca(CN)_2 + 2H_2O \longrightarrow 2HCN + Ca(OH)$$

As usually stated the residue of calcium hydroxide, left after HCN has been liberated is harmless However, in the opinion of Williams [31] this is not quite the case because after liberation some of the HCN is absorbed by the calcium hydroxide and changed to calcium cyanide and water Under any circumstances there is always some calcium cyanide left in the residue which, therefore, must be gathered up and safely disposed off

Williams [31] added, however that

one method of getting around this is to take advantage of the fine powder form of the material and to blow it into the air from which it settles as a fine dust There appears to be no great objection to this in the absence of foods from a safety standpoint it

being inconceivable that anyone would sweep up this and eat it but where foods are fumigated they become inseparably mixed with the fumigant If calcium cyanide is left for several days airing the cyanide content finally becomes so low as to be negligible

Opinions on to what extent calcium cyanide may be used for the destruction of rodents and their fleas vary considerably As maintained in the British instructions[7] against infestations in the open and where rat holes are accessible and easily identified dusting with powders that liberate a poisonous gas can be useful According to the handbook on rat borne disease[17] calcium cyanide should be used in rat control to fumigate burrows and under certain circumstances small enclosed spaces or harbourages (spot fumigation) However as will be shown later, in other areas much more liberal use has been made of the application of calcium cyanide

Calcium cyanide treatment of rat burrows may be done either by pumping in fine dust or by placing heaps of coarser granulations or brands which give off HCN slowly about 6 inches (15 cm) down each hole The treated holes must then be promptly blocked

To get good results with the first mentioned method sturdy and powerful pumps must be used preferably those provided with a cut out device which renders it possible to blow in air after the required amount of dust has been delivered The pumps should be so adjusted that they deliver a known amount of the dust with a given number of strokes for *instance* as recommended by some workers 1 ounce (28 g) with 30 strokes The amounts delivered with a given number of strokes may be ascertained by weighing either the filled pumps or their contents before and after a counted number of strokes has been made

Before actual pumping operations are started the extent of the rat burrows to be treated and their often considerable ramifications must be ascertained *It is of great importance to realize in this connexion that a* large burrow may have openings in two adjoining rooms or even in two adjacent houses Accordingly unless care has been taken to block before commencement of pumping all openings of the burrows to be treated except that to be used for the introduction of the dust a watch must be kept for the escaping of dust and also the bolting of rats not only in the rooms where pumping is carried out but as far as necessary also in adjoining rooms or houses As recommended by Yang et al[13] a mixture of portland cement sand and broken glass should be used for sealing burrows in lime mortar floors and good rubble walls Holes in mud floors and poorly built walls may be sealed with moistened clay or with pieces of sod

In order to prevent a back flow of the dust materials such as clay sod rags or paper must be firmly packed round the nozzle of the pump after its introduction in the hole to be treated After the required amount of dust followed by several blasts of air made with the aid of the cut out device has been pumped in the nozzle is quickly withdrawn and the hole is sealed in the above described manner

The amounts of calcium cyanide used by different workers per rat hole were at variance Thus, Roberts [180] considered it necessary to administer 1 ounce (28 g) of the dust to an average burrow and 2 ounces (56 g) to a burrow with many ramifications Yang et al [213] recommended pumping in 4 g of calcium cyanide when dealing with a rat hole 2 feet (60 cm) in length, and increasing the dosage by 1 g for each additional foot (30 cm) of length Workers in India often found that 1 pound (0 45 kg) of calcium cyanide was sufficient to treat about 60 average burrows or 100 small burrows

In addition to burrows, cracks in walls leading to rat harbourages may be dealt with in the above described manner Thornton [14] recommended applying the nozzle at the lowest point of the cracks and then sealing the cracks with clay as the dust made its appearance

To deal safely with loosely piled stone walls, Thornton [14] advised plastering these on both sides with clay and subsequently pumping calcium cyanide into the holes made in the latter by the rodents

As pointed out in the handbook on rat borne disease, [17] small enclosed spaces inside buildings, such as the boxed in bases of shelving counters and lockers may be spot fumigated ' if there is no danger of the dust going into living quarters or other occupied rooms It was recommended that no place larger than about 100 cubic feet (3 m^3) in volume be treated in this way and that small hand bulb dusters be used instead of powerful pumps Thornton [214] upheld that the latter could be used to treat underfloor spaces with calcium cyanide at the rate of about 2 ounces per 120 square feet (5 g per m) of floor space To reach such spaces holes could be drilled in the floor which, after completion of dusting could be plugged with corks cut flush with the floor level

Williams [3-] established that calcium cyanide could be used safely and efficiently to deal with enclosed rat harbourages such as pipe casings on ships as a preliminary step to general fumigation of the vessels Since the vast majority of such harbourages had a capacity of not more than 10 cubic feet (0 3 m^3) or even considerably less, 4 strokes with a pump delivering 1/12 of an ounce (2 4 g) per stroke were sufficient to dust them adequately

As proved by experiments made in Africa and to a lesser extent also in India, whole huts or small houses may be treated safely with calcium cyanide dust Thornton [14] recommended the following procedure for this purpose

(1) Remove all occupants (people and domestic animals) as well as all foodstuffs
(2) Look for and dust all rodent burrows
(3) Hang all clothes bedding and similar articles on rafters
(4) Spray floor lightly with kerosene emulsion
(5) Cover hut completely with canvas sheets
(6) Pump calcium cyanide into room and thatch using about 1 5 2 pounds per 1 000 cubic feet (28 35 g per m^3) holding pump in inverted position to do this quickly

(7) If possible hold hut under fumigation for at least four hours (or pump in excess of dust)

(8) Remove canvas open doors and windows wait a little before entering

(9) Air clothing bedding etc in the open

(10) If possible keep occupants away until next morning If this is impossible have floor swept before they enter

Hopkins[89] advised applying calcium cyanide only to the rat holes and the thatch of huts in which plague had occurred or was likely to occur, surrounding the huts during this operation with men provided with sticks to kill bolting rats The thatch was then removed and spread out in the sun but the people were permitted to use it for re thatching Roberts[180] recommended the use of pumps provided with long handles to deal with thatched roofs to which the rats in the huts in question were mainly con fined Calcium cyanide was pumped in at a rate of 2 pounds (1 kg) per roof

George[60] studying possibilities for the disinfestation of thatched roofs in India found it sufficient to apply calcium cyanide to the interior of the houses using 12 16 ounces of the dust per 1 000 cubic feet (12 16 g per m^3) with an exposure for 3 4 hours It was necessary however to close tightly doors and windows to seal all small openings with wet mud and to close gaps between the walls and roofs with rolls of gunny bags before pumping was started If the dust was applied in this way hydrocyanic acid gas diffused through the thatch and killed rats and fleas therein

Calcium cyanide has also been used to deal with rodent and flea infesta tions in other sites e g with grain and haystacks and with railway trucks carrying produce Its use for the purposes of grain disinfestation in general will be discussed later in connexion with the problem of flea control

Since HCN is slightly lighter than air (specific gravity 0 9348) it is well to deposit calcium cyanide at the base of the objects to be disinfested or when treating objects such as cracks in walls or grain and haystacks to start pumping at the lowest point and gradually to move the nozzle of the pump upwards

Though generally speaking it is not necessary to adopt elaborate precautions as indicated in the case of HCN fumigation when working with calcium cyanide the following safety rules must be strictly followed

(1) The tins containing calcium cyanide must be tightly closed as soon as the necessary amount of dust has been withdrawn and must be stored in a dry and safe place

(2) Preparations for calcium cyanide application (filling and testing the pumps etc) must be made in the open or in open sheds Unless the latter offer protection operations should be suspended if there is a high wind In a slight wind the operators should stand to windward of the tins and pumps so that any dust blown out is carried away from them

(3) It is inadvisable to dust in wet weather or to deal with burrows in the open when the ground is wet

(4) Calcium cyanide application should be made by teams of at least two workers so that, should one inhale HCN, the other, or others, can take him into the open and assist him

(5) Generally, it is not necessary to use gas masks when handling and distributing calcium cyanide but they should be worn preferably for spot fumigation in rooms Also, as suggested by Hopkins [89] it is well if each team is provided with one gas mask to serve in emergencies

(6) Before operations are commenced in buildings, all inmates including domestic animals must be sent out and must be kept away until it is safe for them to re enter the premises

(7) All foodstuffs and as far as possible, all water supplies ought to be removed from the premises to be treated

Opinions as to how long the people and domestic animals ought to be kept away from premises treated with calcium cyanide vary considerably Some workers considered a few hours ventilation sufficient while others advised that the premises ought not to be re occupied for 1 3 days after application of the dust Generally speaking as long as good facilities for ventilation and airing of objects dusted exist, an evacuation until the morning following treatment ought to be ample As recommended by Yang et al [213] one might in emergencies spray chloropicrin at the rate of 50 ml per 1 000 cubic feet (28 m³) in the endangered rooms so as to make sure that the people do keep away

Since after calcium cyanide application the rodents often succumb in their burrows not rarely decomposition of the carcasses of these animals produces bad odours in the houses treated The methods mentioned earlier in this study may be used in such cases To seal all cracks in the walls of simply constructed houses may be helpful as well

The following methods for treating victims to poisoning with cyanides are given in *Rat borne disease prevention and control* [17]

Any case of cyanide poisoning must be treated rapidly Persons using this fumigant should always carry amyl nitrite ampoules a sodium nitrite solution of 0 3 grams in 10 cubic centimeters of water and a sterile 10-cubic centimeter syringe and needle Until the sodium nitrite solution can be injected INTRAVENOUSLY by a physician the patient may be tided over by holding a broken amyl nitrite ampoule under his nose for 30 seconds in every 2 minutes The 10-cubic centimeter sodium nitrite solution should be injected by a physician as soon as possible the injection being slow and taking 3 to 4 minutes Follow this injection by another slow INTRAVENOUS injection of the entire 50-cubic centimeters of sodium thiosulfate solution over a period of 10 minutes Call a physician at once but do not wait for him to administer the amyl nitrite If breathing stops apply the prone pressure method of artificial respiration immediately

The results obtainable with calcium cyanide may be evaluated as follows

(1) It would seem at first glance that application of calcium cyanide is particularly advantageous because this chemical is endowed with pulicidal as well as rodenticidal properties Actually, however, reductions in the incidence of either rodents or fleas obtainable through single calcium cyanide applications are not drastic enough to be of lasting value A considerable minority sometimes even about half of the rats in the localities treated in this way remains unscathed with the result that increased breeding of these survivors rapidly restores the former population level of the animals The reduction in the rodent flea incidence effected through calcium cyanide dusting is even more temporary mainly because it is impossible as a rule to deal effectively with the aid of this procedure with the flea nurseries in deeply situated rodent nests (Ganapathy [59])

It follows that the only way in which it is possible to keep the rats and their fleas at a permanently low level through application of calcium cyanide alone is to repeat the campaigns at short intervals as it is usually recommended every three months Quite possibly by continuing such quarterly campaigns for some years one might bring about a very marked reduction of the rats or might even well nigh eradicate them However the effort and expenditure involved would render this impracticable

Even single applications of calcium cyanide have been found to be of definite value in so far as they were apt to limit or if commenced early in the outbreaks even to cut short the spread of plague However results obtainable with single applications of DDT are markedly better than those with regularly repeated calcium cyanide applications Thus comparative tests made in the Nilgiris District of Madras State India in 1948 9 showed as summarized by Wagle & Seal [27]

" that DDT is very effective in keeping the general flea index below 1 for nearly 16 months after a single application while with repeated cyanogas [calcium cyanide] fumigations in Coonoor town the monthly flea index remained quite high Again while plague occurred in 45 out of 460 village units regularly fumigated with cyanogas every 3 or 4 months all the 90 village units treated with DDT escaped infection during the same period "

While therefore there can be not the least doubt that DDT rather than calcium cyanide must be used for plague control in general the latter remains of some value for certain specific purposes particularly for grain disinfestation

Methyl bromide

Methyl bromide (CH_3Br) having a boiling point of 3 56°C is at ordinary temperatures a colourless gas which is heavier than air (specific gravity according to Frear [86] 3 20 at 20°C) In the concentrations used for fumigation the gas is not inflammable It is easily liquified and is therefore usually distributed in this form in cylinders

Though not necessarily harmful even in relatively high concentrations, methyl bromide is apt to exert a delayed toxic action on man Workers handling this chemical should therefore, wear suitable masks, the more so because the sweetish odour of the gas is not pronounced

As recently stated by Gracie,[69] methyl bromide was used successfully in Great Britain to deal with considerable mouse infestations in hangars where bagged grain was stored For this purpose the piles of bags were sealed under balloon fabric and methyl bromide was injected The whole operation (sheeting fumigating, and ventilating) took three days

Sulfur dioxide

Sulfur dioxide (SO_2) generated by combustion of sulfur in pots or with the aid of a special apparatus (Clayton machine) or released from cylinders containing the chemical in liquid form has been amply used in the past for ship fumigation and is still utilized for this purpose to quite some extent in ports where it has not been found possible thus far to apply more satisfactory methods

Fumigation of rat burrows or other enclosed spaces with sulfur compounds has also been practised in some countries In India for instance, sulfur containing candles called *neem battis* or *bhoosa battis* were utilized to fumigate rat burrows As was to be expected, Yacob[21] found these candles markedly less effective than calcium cyanide

Concerning the fumigation of ships one must agree with Williams[31] that SO_2 is the only nearly safe fumigant known because it causes severe irritation if present in the air in concentrations far below the fatal level However, as rightly pointed out by Hirst[86] this property is a drawback as well as an advantage, because the rats as well as man get early warning of the presence of the gas and have therefore time to seek refuge in enclosed spaces such as covered bilges, into which the gas could only penetrate with difficulty

A still greater drawback is that SO_2, being a very heavy gas (specific gravity, 2 26), has slow powers of penetration, so that it has to act for 6 hours at least to be effective To make matters worse SO_2 if used for this length of time and in the required concentration of 3%, tarnishes metals and damages cereals and other goods so that it can be used in empty holds only (Hirst[86] Wu[38])

It will be gathered that the method of fumigating ships with sulfur dioxide has, indeed little to recommend it and one must wish that it will be discarded as soon as possible

Other methods

In addition to the above described methods the following procedures used for the destruction of commensal rodents deserve mention

Electrocution

Besides electricity charged traps other means have been used to electrocute rats e g fixing live cables in sewers or suspending baits between electric wires from which the insulation has been removed for a short distance (Rucker, quoted by Wu [39])

Use of sticky substances

Flat boards coated with highly sticky substances have been placed in the runs of rats or mice in order to entangle the animals Flat trays filled with a mixture of linseed oil and resins and provided with baits placed in the centre of the trays on pieces of cardboard have been used in the same way However as pointed out in the handbook on rat borne disease [217] the sticky substances tend to run in hot weather and to solidify in cold weather Moreover, these procedures are rather expensive to use and though fairly effective against mice are relatively inefficient in the case of the rats

Khan [101] claimed to have obtained good results when placing in the openings of rat burrows or on rat runs sticky materials such as molasses which had been lightly sprinkled with dried and powdered datura bella donna leaves and copper sulfate or with sodium antimony tartrate powder

Flame throwers

Donovan & Hopkins [45] found that flame throwers (fire torches) hitherto used only in industry and in war were efficient for the purposes of rodent and flea control They recommended in particular one type of torch with a four gallon tank connected with a burner coil by a flexible rubber hose Though the flame had a very high temperature Donovan & Hopkins claimed that the torch could be used on wood bamboo or cane surfaces without setting fire to them

As stated by these two workers the flame throwers were found effective

" for burning off vegetations from ditch banks killing rats and fleas in burrows burning abandoned rat nests with their possibly infected fleas in mud walls stone fences adobe walls and other shelters killing fleas within infected houses killing rats fleas spiders bed bugs ticks cockroaches lice and other vermin in cracks and crevices of walls and floors in routine sanitation work in dwellings restaurants warehouses and other premises killing rats in sewers and igniting brush fences (a favorite rat harbor) garbage and rubbish piles "

The value of flame throwers for plague control work was confirmed by Sáenz Vera (1943) [183] who however, stated that it could be used only in sites where no fire hazard existed—an opinion shared in the handbook on rat borne disease [217] Barreto & Castro [13] considered the use of flame throwers less effective and more dangerous than that of calcium cyanide It seems also significant that Saenz Vera in a further publication [184] speaks only of the use of the latter and not of that of flame throwers

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Rat proofing

The following methods may be used for the purposes of rat proofing which, as broadly defined by writers such as Bowdoin & Boston[19] aims at "the separation of rat and man"

Exclusion

Exclusion methods by which the ingress of rodents into houses or other structures is prevented, while being the most effective of all rat proofing procedures are also those most difficult to apply Their implementation requires not only the availability of adequate materials and funds but also a high degree of specialized technical knowledge And even if all these facilities are available it may be difficult or even impossible to apply the exclusion methods effectively in the case of huts or houses of primitive construction *Still*, one cannot help feeling that the defeatist attitude the presence of which in rodent control work in general is deplored by Barnett[9] is particularly conspicuous in the field of rodent exclusion The expenditure for work of this nature is not rarely overrated Moreover though it may be impossible for financial or technical reasons to rat proof all existing buildings, this ought not to detract attention from possibilities of dealing effectively with some of them, particularly those in which grain or other foodstuffs are accumulated, or from possibilities of making new buildings impervious to the rodents—a task which can often be achieved satisfactorily at a moderate increase of the building cost

Essential measures for rendering buildings inaccessible to rodents particularly to rats are the following

1 To provide solid floors preferably concrete floors and/or to install L shaped curtain walls the lip of which should lie at least 2 feet (60 cm) deep and should protrude outwards for one foot (30 cm) or better 2 feet (60 cm) As stated by Porges,[12] such curtain walls which may be built around existing buildings protect these against the rats in the absence of foundation walls or of insufficiently deep foundations The curtain walls are also useful in the case of structures built on supports not high enough to prevent rats from gaining entrance

2 To use what Bowdoin & Boston[19] call 'vent stoppage', i e, the blocking of all openings ½ inch (50 mm) wide or wider through which the rodents can gain entrance into the buildings For this purpose a careful search must be instituted for holes made by the rodents, and these must be sealed preferably with cement containing pieces of broken glass Man made openings, such as those through which pipes or conduits pass, must be closed with the aid of cement or metal sheathing Wooden sills and doors at ground level should be protected against gnawing with the aid of sheet metal Windo ss than 4 feet ( ) off the ground must be screened with a rong wire If *R rattus* are present,

the same ought to be done in the case of the windows in upper storeys unless rat guards have been placed on all conduits and pipes so as to prevent the animals from climbing up. Attention must also be paid to the possibility of rats jumping into such windows from poles or trees standing too near the houses.

Great attention must also be paid to the openings of sewers which must be adequately protected with solid grids.[b] The improved buildings must be periodically inspected so as to ensure that the barriers created against the rodents are kept in good condition.

Smaller buildings may be effectively protected against the access of rodents by placing them on supports at least 18 inches (45 cm) high and provided with rat guards (metal collars) extending at least 9 inches (23 cm) from the supports. Buildings protected in this manner or even covered platforms placed on higher poles provided with rat guards are suitable for the storage of foodstuffs, particularly of grain.

Rat guards placed on cables mooring ships to the shore are an important means of protecting vessels against the access of rats, provided that the rat guards are of a sufficiently large size and carefully adjusted. Unfortunately, however, it is not easy to fulfil these two desiderata. Denney[39] carrying out experiments with various patterns, found that the *R. r. alexandrinus* used for this purpose was finally able to pass over guards with a diameter of 35 inches (90 cm) as used in the Panama Canal Zone. As shown by recent tests recorded by Stock,[10] the electrically charged rat guards recommended by some workers may also prove unsatisfactory.

Whole settlements as well as individual houses or blocks of houses may be protected against the ingress of rodents. Thus Vincke & Devignat were successful in keeping villages in the Belgian Congo, in which deratization had been carried out, free from re-infestation by surrounding these settlements with trenches 18 inches (45 cm) wide and about 40 inches (1 m) deep with vertical sides. Rats which fell into these trenches could not jump out but occasionally escaped through drainage channels. However, care was taken to have these run in directions away from the villages.

Abolition of shelterage

Methods of abolition of shelterage or of "internal" rat proofing, as these procedures are sometimes called, are of great importance in so far as (*a*) their application is less tedious and less expensive than that of exclusion methods and (*b*) they can be successfully used in buildings of a primitive construction from which it would be difficult or even impossible to exclude the rodents.

The question of whether "internal" rat proofing should precede or should be followed by eradicative procedures has been differently answered

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[b] Adeq ate d t ls for the mpleme tatio of the e proc d es will be fo nd n p mphlets p bl hed by H lse dorf a d S l er et al as well as in *R t b f e p e ll n a d o l*

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Smaller buildings may be effectively protected against the access of rodents by placing them on supports at least 18 inches (45 cm) high and provided with rat guards (metal collars) extending at least 9 inches (23 cm) from the supports. Buildings protected in this manner, or even covered platforms placed on higher poles provided with rat guards, are suitable for the storage of foodstuffs, particularly of grain.

Rat guards placed on cables mooring ships to the shore are an important means of protecting vessels against the access of rats, provided that the rat guards are of a sufficiently large size and carefully adjusted. Unfortunately, however, it is not easy to fulfil these two desiderata. Denney[39] carrying out experiments with various patterns found that the *R. r. alexandrinus* used for this purpose was finally able to pass over guards with a diameter of 35 inches (90 cm), as used in the Panama Canal Zone. As shown by recent tests recorded by Stock,[10] the electrically charged rat guards recommended by some workers may also prove unsatisfactory.

Whole settlements as well as individual houses or blocks of houses may be protected against the ingress of rodents. Thus Vincke & Devignat were successful in keeping villages in the Belgian Congo, in which deratization had been carried out, free from re-infestation by surrounding these settlements with trenches 18 inches (45 cm) wide and about 40 inches (1 m) deep with vertical sides. Rats which fell into these trenches could not jump out but occasionally escaped through drainage channels. However, care was taken to have these run in directions away from the villages.

Abolition of shelterage

Methods of abolition of shelterage or of "internal" rat proofing, as these procedures are sometimes called, are of great importance in so far as (*a*) their application is less tedious and less expensive than that of exclusion methods and (*b*) they can be successfully used in buildings of a primitive construction from which it would be difficult or even impossible to exclude the rodents.

The question of whether "internal" rat proofing should precede or should be followed by eradicative procedures has been differently answered

[b] Adequate details for the implementation of these procedures will be found in pamphlets published by Holsendorf ... and Silver et al. ..., as well as in *R ... b ... l ... a ... p ... t ... a ... d ... t ...*

by different workers, but a majority is in favour of first reducing the rodent populations by the application of eradication methods and then resorting to rat-proofing Calhoun [71] was not, in general, in favour of this policy because, in his opinion, ' sanitation procedures which involve the limitation of availability of food or harborage for rats are unlikely to increase more than temporarily the number of rats in adjoining areas " He admitted, however, that when dealing with groups of rodents known to harbour human diseases, killing techniques had to be applied first in order to avoid a wide and rapid dissemination of the disease carrying rats

The methods to be used for internal rat proofing must be adapted to the species of rodents present If Norway rats alone are found, main attention must be paid to the abolition of shelterages situated underground and on the ground level of the buildings If *R rattus* are present, great care must also be taken to deal with the dead spaces created by double walls and false ceilings and to replace, whenever possible, thatched roofs by those made from solid materials

If, as was the case in Java bamboo poles are used for the purposes of house construction, these must be properly sealed to prevent the rats from sheltering in these hollow fixtures However at present wooden beams are used for house construction or house improvement in Java [1]

It is essential to deal with the above mentioned and similar structural rodent harbourages [c] in as radical manner as possible making in particular determined efforts to reach and destroy the rodent nests At the same time however, due attention must be paid to the feelings and needs of the people during the performance of drastic operations of this kind For instance, they often insist upon having double walls and false ceilings not merely for the sake of attractiveness but as a means of protecting themselves against cold or heat These needs for insulation should not be disregarded therefore, but should be met with in an adequate manner Thus, insulating materials such as boards or mats formerly used for the creation of double walls should be nailed directly to the main walls of the houses If it is necessary to retain false ceilings, sufficiently large openings should be provided in them to prevent the rodents frequenting the spaces above such partitions being unobserved and also in order to make it possible to inspect and clean these spaces

Articles of furniture such as cabinets and chests of drawers, should be placed on supports and should preferably not be placed directly against the walls so that the spaces under and behind them can be inspected and cleaned

Great attention must be paid to the abolishment of temporary harbourages created by accumulations of useless materials and by the storage of fuel or other bulk supplies The former, which often offer excellent nesting

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[c] See section on rat harbourage in chapter 6

facilities to the rodents must be removed and burnt Necessary bulk supplies should be staked on supports high enough to prevent the rodents from sheltering under them and to facilitate house-cleaning

Abolishment of shelterage possibilities for rats on board ship instituted through the pioneer work of American experts particularly of Grubbs & Holsendorf [6] has given the most excellent results The systematic use of these procedures renders it possible to keep the vessels free from rats to such a degree that they can be exempted from periodical fumigation

Food protection

It seems legitimate to deal with the methods used for the protection of food supplies from commensal rodents in the course of the present disquisition because as they constitute an important means of separating these animals from man they form part and parcel of the methods of rat proofing as defined above

Food supplies for human consumption kept in individual houses can be safeguarded by keeping them in solid covered containers for instance in earthenware vessels as they are often available in rural areas—which may be covered simply by stone plates Another satisfactory method is to use baskets or other suitable means to suspend the food supplies from the ceilings in such a manner that the rodents cannot reach them

Even if adequate care is taken of the stored food supplies the commensal rodents are often able to find ample nourishment in containers used for the preparation and consumption of meals such as pots bowls or plates which have not been promptly cleaned after the food has been partaken Indeed in less affluent households in which stored food supplies may be scanty the food remnants in uncleaned containers left standing during the night often form the main source of food supply the rodents find in the houses It is therefore of great importance to remedy this situation by cleaning all food containers soon after the meals particularly after the evening meals and by safely disposing of the food residues

Great care must also be taken to prevent access of the commensal rodents to food supplies particularly to cereals kept for feeding the live stock If not bulky such supplies may be safeguarded in the same way as those for human consumption Large amounts should be kept in solid bins or in cribs or on platforms raised on supports which are provided with rat guards

The supplies in small food shops may be protected against the access of commensal rodents in the same manner as those kept in residences Establishments in which foodstuffs are stored or handled in bulk should be protected with the aid of exclusion methods as well as those of internal rat proofing

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Great care must also be taken to prevent access of the commensal rodents to food supplies, particularly to cereals, kept for feeding the live stock. If not bulky, such supplies may be safeguarded in the same way as those for human consumption. Large amounts should be kept in solid bins or in cribs or on platforms raised on supports which are provided with rat guards.

The supplies in small food shops may be protected against the access of commensal rodents in the same manner as those kept in residences. Establishments in which foodstuffs are stored or handled in bulk should be protected with the aid of exclusion methods as well as those of internal rat proofing.

Sanitation

Adequate sanitation methods, implemented within the houses and compounds as well as on a community-wide scale, are of great importance for the proper control of the commensal rodents All household refuse must be deposited in solid covered receptacles Back yards and alleys must be kept clean and free from accumulations of waste products which if considerable, are apt to offer shelter as well as food to the rodents When dealing with emergencies in settlements having no, or no adequate, social services, the plague workers must also make temporary arrangements for the proper collection and disposal of the refuse It is better under such circumstances to burn the latter or to bury it at a depth of at least three feet (1 m)

Public health propaganda and education

It is no exaggeration to say that full success in the fight against the commensal rodents can be obtained only when it is possible to make the people conscious of the harm caused to them by these animals and thus to enlist their sympathy for and help in, the work

Though the choice of the methods to be used for this purpose depends upon the local conditions, it is advisable under all circumstances that lessons dealing with the damage caused by the rodents and the procedures for their control be made part of the school curricula

When trying to reach the adults emphasis ought to be laid upon publicity through illustrated posters, newspapers and broadcasts rather than upon the distribution of leaflets or pamphlets Illustrated lectures particularly the demonstration of films may have a still greater appeal Temporary or permanent exhibits, particularly if held in model houses showing the various means of rat proofing, are also of considerable value It may likewise be useful to interest the people in anti rodent work by arranging for clean liness days or weeks during which volunteers are set to work under supervision to deal with accidental rat harbourages and insanitary conditions in general

Perhaps more important still than these propaganda methods are informal talks given to the inhabitants of houses visited for the purposes of surveys and control work The periodical inspections of the buildings, which must be made in all localities where anti rodent programmes have been implemented, offer good opportunities of keeping the people interested in the work

To what extent the confidence and co operation of the population can be obtained is well illustrated by experiences in Java where it has been possible to continue the house improvement work, instituted on a large scale under Government auspices in 1914 through the voluntary efforts of the people

Administrative action

In rodent-control work as in anti plague work in general it is preferable to use methods of persuasion rather than administrative action to obtain the goodwill and help of the public It is however, advisable to make two exceptions to this rule

(1) After a careful consideration of the local conditions and the easily available building materials, regulations for the rat proof construction of new structures ought to be promulgated and building permits ought to be issued only if the plans have been drawn up in accordance with these specifications

(2) The implementation of approved rat proofing methods ought to be made obligatory for the establishments in which goods attractive to the commensal rodents particularly foodstuffs are stored or handled in bulk However, all possible efforts ought to be made to render the execution of such work as easy as feasible for instance by supplying the necessary materials at cost price from the stocks of the rodent-control or anti plague service

Planning and co ordination

Disappointing though it often is that no or but limited use can be made of drastic rat proofing procedures even less satisfactory methods if applied not at the spur of emergencies but according to a well-considered long range plan are apt to give good results It is of great importance in this connexion to link up the anti rodent work carried out for the purposes of plague control with that of other agencies which are or ought to be interested in the fight against these animals Indeed though the plague workers must be ready to take the action dictated by emergencies by rights rodent control work should form a permanent part of the programmes adopted for the improvement of environmental sanitation in general

Comparative value of the methods of commensal rodent control

Unanimous agreement has been reached that the methods of " building out the rodents through the application of rodent proofing procedures are by far preferable to killing campaigns which may give rapid results but in order to prove effective in the long run have to be carried out periodically or—in the case of the anti-coagulants—have to be continued An emphatic resolution to this effect was adopted by the WHO Expert Committee on Plague at its second session in 1952 [37]

Nevertheless the methods of directly attacking the rats are not only indispensable for the fight against plague but are also of great [illegible] value in so far as (*a*) owing to the nature of the structures to be dealt with or for financial or administrative reasons it is often difficult or even impos

sible to take large scale advantage of rodent proofing methods, and (b) even if the latter can be used it is necessary, as a rule to decimate the rodent populations before or, as some workers advocate, immediately after exclusion or internal rat-proofing procedures have been implemented It is therefore, often indicated to utilize a judicious combination of proofing and eradicative methods for the fight against the commensal rodents

Control of Wild Rodents

As described by Wu Lien teh [739] and by Wu Lien teh & Pollitzer, [10 211] large scale campaigns aiming either at the destruction of wild rodents round plague threatened settlements or at eradication of these animals in more extended areas have been carried out in South Africa, south east Russia, and the western parts of the USA, particularly California Besides shooting the wild rodents—a procedure found successful in the USA—the methods mainly used against these animals were

(a) Poisoning with strychnine or sometimes with other rodenticides especially arsenic

(b) Fumigating the rodent burrows with carbon disulfide and, in south-east Russia also with chlorine or chloropicrin As claimed by Mamontov & Kolpakova [1] the introduction of calcium cyanide into the burrows of the sisel *Citellus pygmaeus* also gave satisfactory results

In South Africa ample use was also made of destroying the wild rodent burrows with the aid of agricultural dynamite Distribution of "virus", experimented with there as well as in south east Russia, proved unsatisfactory

As claimed by Ioff [96] in 1941, widespread anti plague operations, including campaigns against the *Citellus* populations led to a liquidation of the epizootics in the Caucasian steppes where in 1937, despite thorough and widespread investigations, no plague foci could be discovered Since no further information on these areas is available, it is not possible to say whether or not this success was really permanent Meyer,[1-5] commenting on Ioff's report, professed scepticism pointing out that optimistic claims made in 1914 in regard to the eradication of plague in California had been disproved in a most disappointing manner by further experience In fact as has been stated in chapter 1, plague not only persisted there but gradually became entrenched among wild rodent populations in neighbouring states Recently reviewing this situation Link [113] came to the conclusion that the vastness of the area involved makes it impracticable even to think about the eradication of plague in these rodents even if one could forget the illuminating story connected with attempts to eradicate plague in the California ground squirrel Consequently, while closely watching the plague situation in the wild rodents workers in the USA now lay emphasis upon protecting urban settlements against inroads of the infection through the control of the commensal rodents in these places However,

as pointed out by Meyer[13] satisfactory though this policy is as far as it goes work of this kind is unable to liberate the rural communities from a constant hazard which is thus far, unfortunately, unrecognizable and immeasurable

An identical policy has now been adopted in South Africa where, according to Davis,[37]

"the main principle of control is to keep the sylvatic foci under surveillance and to eliminate them on a small scale when practicable but to concentrate upon preventing the infection from becoming established in close proximity to man"

VECTOR CONTROL

DDT

2 2 bis-(*p*-chlorophenyl) 1 1,1 trichloroethane commonly called DDT in abbreviation of its generic name dichlorodiphenyltrichloroethane is a comparatively slow acting but most effective insecticide because (*a*) mere contact with minute amounts of this compound is apt to kill insects such as lice mosquitos and fleas inexorably though gradually and (*b*) surfaces on which DDT has been deposited retain this killing power for considerable lengths of time not rarely for many months This property of prolonged residual action makes DDT particularly useful for the control of plague and flea borne typhus as well as of other insect borne diseases

As shown by ample experiences DDT is fully effective for the control of the common rat flea species (*Xenopsylla cheopis Nosopsyllus fasciatus* and *Leptopsylla segnis*) and as shown by Sharif[193] also for that of *X astia* Observations in the southern part of the USA suggested that *Echidnophaga gallinacea* was less amenable to control with *DDT* than the above mentioned species and this was confirmed by Macchiavello[119] in Tumbes Peru However as pointed out with much reason by Davis[3] these sticktight fleas possibly escape the action of DDT merely because they are wont to bury their head and thorax into the skin of the rats and are thus little exposed to the insecticide Hill & Morlan[84] ascribed the erratic results they obtained with DDT dusting in the case of these fleas to the care they took in distributing the insecticide only in places where chicken could not come in contact with it In fact Eads[51] obtained satisfactory control of *E gallinacea* when freely applying 10% DDT dust to a large hen house

Boiron[16, 17] noted that *Pulex irritans* which had recently replaced *Synosternus pallidus* in the houses of Dakar Senegal was not in contrast with the latter flea amenable to control with DDT and therefore raised the question of whether a resistance to this insecticide might have enabled

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P. irritans to become predominant. However experiences made by other workers, by Davis [35] and Macchiavello [119] for example, showed this flea to be fully sensitive to the action of DDT.

General agreement exists that the control obtainable with DDT dusting in the case of the tropical rat mite, *Liponyssus bacoti*, and the rat louse *Polyplax spinulosa*, is more or less incomplete. Nicholson et al. [149] applying for the purposes of combined typhus and malaria control, DDT in spray form to the walls and ceilings of the houses as well as to the floors and rat burrows obtained more encouraging results with *L. bacoti* and believed therefore that this mite was not affected by DDT dust on account of its climbing habits.

According to Wiley & Fritz, [33] DDT dusting gave satisfactory results in the case of two other rat mites—*Echinolaelaps echidninus* and *Laelaps nuttalli*.

It is distressing to note that recently some evidence has become available to show that flea populations as well as groups of certain other insect species may acquire a resistance to DDT after repeated applications of this insecticide in the localities in question. Kilpatrick & Fay [106] recorded in this connexion that in 1949 several reports had been received of the persistence of numerous *Ctenocephalides felis* after as many as three applications of 5% DDT dust in the infested premises. The two workers made experiments therefore to see whether *X. cheopis* stocks reared in the laboratory could be made resistant to DDT by continued exposure of the survivors of earlier tests to further doses of the insecticide. It was possible to show in this way an increased DDT resistance of the fleas through the F_3 generation, the mortality decreasing from 65% in the parent generation to 32% in the F_3. Since no further increase in resistance was shown in further tests through the F_7 generation, Kilpatrick & Fay did not ascribe practical importance to these experimental findings. Good (quoted by Hess [83]), who had found some evidence of the development of a DDT resistance by *X. cheopis* under field conditions, likewise assumed that such resistance as may have developed is not sufficient seriously to affect operational programs where 10 per cent DDT dusts are used to control this species. However Saenz Vera (Ecuador) [185] in a report submitted in 1952 to the WHO Expert Committee on Plague [37] stated that

> Unfortunately since 1950 we have noticed that in certain areas considered as plague foci and systematically treated with DDT, a number of fleas survive the application of the insecticide.

Application of a new batch of DDT in these areas gave no better results while the formerly used batch was found to be fully effective in a hitherto untreated area.

When visiting Brazil in 1952, Sáenz Vera was informed that in rural areas of the Pernambuco State as well new applications of DDT had

little effect He added in a further (personal) communication that the fleas involved were *X cheopis P irritans N londiniensis* and *Polygenis* species

Action has been initiated by WHO to study further this matter

It was noted by some observers that dusting with DDT for the purposes of plague or typhus control exerted a lethal action not only on the fleas but also to some extent on the rodents Thus Machiavello [119] describing his work in Tumbes mentioned that rats (*R rattus*) dusted with a heavy dose of DDT (1 2 g of a 10% mixture) may be poisoned and die because they regularly and carefully lick their fur Referring again to this matter in 1949,[1 1] he stated that the average lethal dose of DDT dust for these rats equalled 0 5 g of the pure substance but that the range of toxicity varied from 0 05 1 g The action of the insecticide did not seem sufficiently reliable to replace the usual rodenticides in the field

However, the use of 5% DDT dust for the control of mice and that of 50% dust for the control of rats and *Meriones* was recommended by Sergent & Sergent [191 19] Likewise Wanson & Camphyn [2 9] suggested that 50% DDT dust might be used for the destruction of rats and gerbils

It is generally agreed that the distribution of 5% or 10% DDT dust may be used with advantage for the destruction of commensal mice It seems not advisable however to use 50% DDT to destroy larger rodents which may be dealt with more effectively and economically by other methods

Technique of application

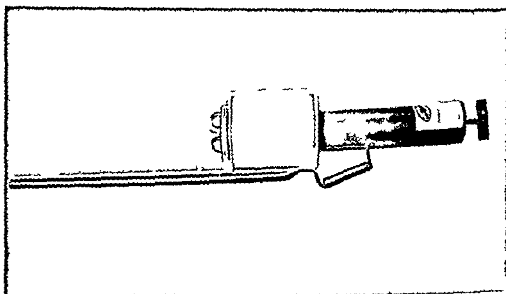
DDT-containing dusts or fluids are used for the control of plague and flea borne typhus either singly or in combination

To apply DDT by dusting it is necessary to obtain it from the producers ready made in 10% strength because it is not possible to prepare such formulations from concentrates without special machinery If it is desired to use 5% dust the 10% formulations may be mixed as needed with equal parts of inert materials such as talcum powder or kaolin Flour has also been utilized for this purpose Care must be taken that the inert material added has an adequately fine particle size

Claims have been made by some workers that 5% DDT dust gives as satisfactory results in flea control as the originally used 10 % formulation However Good [68] stated that in his experience 5% DDT dust was definitely less satisfactory for *cheopis* control than 10% dust This opinion was endorsed by a statement made in 1951 [4] according to which " on 5 percent DDT dust projects rats from premises dusted more than 1 year previously showed a high percentage of infestation and an average per rat examined of 0 7 as compared to 0 3 for comparable periods of 10 percent DDT dust projects As noted in a 1952 report [174] " most operators have preferred to use the 10 per cent formulation particularly where it was desirable to control other ectoparasites more resistant to DDT for example the cat flea "

For these reasons as well as in view of the recent observations on DDT resistance quoted earlier, the present writer is definitely of the opinion that, in order to work with adequately high concentrations of the insecticide, the use of 5% dust formulations should be discontinued Essential though it is to be economical in plague work, one must beware of false economies as they are sometimes practised in DDT application in particular

FIG 39 HAND DUSTER WITH LONG METAL NOZZLE



In addition to the hand dusters with long metal nozzles (see fig 39) supplied by the producers in distributing DDT dust it is necessary to use (*a*) foot pumps of the pattern used for calcium cyanide distribution provided preferably with an additional dust reservoir tank of 5 pound (2 25 kg) capacity, (*b*) hand operated rotary blower dusters, and (*c*) hand shaker dusters —both large ones, consisting of a box made of sheet metal with a removable wire gauze net or a perforated removable metal screen at one short side and a stout handle on top, and small ones (capacity ¾ pound or 0 33 kg), cylindrical in shape, with a removable perforated lid and provided with a handle 3 to 6 feet (1-2 m) long[118 417] If no shaker dusters are available these can be improvised by punching holes into the lid of a tin can, preferably one with a screwed on lid

DDT solutions can be simply prepared by dissolving technical grade DDT in kerosene at the rate of 5 g to 100 ml of the solvent or by taking 6 7 ounces of technical DDT per gallon of kerosene Other solvents, for instance camphor oil, may be substituted for kerosene[d]

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[d] A specification for technical DDT has been established by the WHO Expert Committee on Insecticides (*Insecticides manual of specifications for insecticides and for spraying and dusting apparatus* specification WHO/SITECH/1 p 1)

DDT emulsions may be made by dissolving technical DDT in a good solvent and then adding soap or another emulsifier so as to render further dilution of the fluid with water possible However to obviate the tedious process of preparing emulsions in the field the fluid DDT concentrates supplied by the producers—which merely require addition of water to obtain a 5% formulation—may be utilized Dry wettable DDT products, containing an emulsifier in powder form, have recently become available from these suspensions can be made by simply adding enough water to obtain a 5% strength of the insecticide

Various models of sprayers ranging from small hand driven patterns to large pressure sprayers, have been recommended for the distribution of fluid DDT preparations Whatever kind of sprayer is used it is essential to apply the material as a coarse spray and not as a fine mist Nozzles which give a fan shaped spray are preferable (Bishopp [1])

Generally speaking DDT emulsions and suspensions, because they leave a more massive deposit are preferable to the solutions for flea control work The inhabitants of well appointed houses may object to these deposits On the other hand however the smell of the solutions is objectionable unless an odourless solvent is available and a fire hazard may be created by their distribution

A further important reason why for the purposes of flea control DDT in dust form is preferable to fluid preparations is that the sprayed materials remain fixed to the surfaces on which they have been spread while dust deposits are apt to become transferred to the fur of the rodents and to be carried by the latter to their burrows and nests The only drawback of dusting is that the deposits on the floors may be swept away by the people Nicholson et al [119] suggested for this reason that such vulnerable sites should be sprayed with fluid DDT preparations while dust ought to be used in the other parts of the premises to be treated

It is generally recommended that fluid DDT preparations be used in a strength of 5% and applied at the ratio of about 40 ml per square metre or of one gallon per 1 000 square feet so as to obtain deposits of 200 mg of concentrated DDT per square foot In sites needing less intensive treatment half of these dosages may suffice

Though larger or lesser dosages of 10% DDT dust have been used by some workers it is usually considered adequate to apply the dust at the ratio of 2 3 g/m or 200 300 g (7 10 ounces) per 1 000 square feet It is however important to note that Davis [35] and most other workers in the USA maintain that the quantity of DDT used ought to depend upon the degree of rat infestation rather than upon the size of the sites to be treated

In order to apply DDT efficiently for the purposes of rodent flea control it is essential to make preliminary inspections of the premises to be treated so as to ascertain what species of rodents are present and to

assess the degree of infestation Whenever possible preliminary rat trapping ought to be done in the localities to be dealt with so as to establish the flea indices and/or the percentages of flea infestation

Most workers insist that, in order to obtain fully satisfactory results, it is necessary to apply DDT not only to all rat-runs on the floors as well as in elevated positions (e g, on rafters), but also to the rodent burrows and other enclosed or semi enclosed harbourages, such as dead spaces under floors or between double walls, which may be rat and/or flea infested

A sound principle now adopted in the USA is that, when applying DDT to the rat runs, stress ought to be laid upon 'patch dusting" rather than upon uniform distribution of the dust in thin layers so as to increase the chances that the rodents, after having come in contact with the deposits, spread the dust over their fur when preening themselves and carry it into their burrows

Patches of DDT dust ought to be placed with the aid of shaker-cans (a) in the first line round the entrances of rodent-burrows or other holes made by the rodents on level surfaces, and (b) on rat runs both on the floors and on rafters or other elevated surfaces The quantity of the dust to be used for individual patches depends upon the degree of rodent infestation present As stated in *Rat borne disease prevention and control* [17]

When infestation is light only a relatively thin layer, enough to be seen is needed For a moderate amount of rat evidence a patch 1/32 inch [0 8 mm] thick is sufficient Heavy infestations demand patches of 1/4 of an inch [6 4 mm] or more"

The rings of DDT dust made round rat holes ought to have a diameter of 6-12 inches (15 30 cm) DDT patches on rat runs, which should be placed at intervals of 10 15 feet (3 4 5 m) preferably in narrow parts of the runs, should be 6 inches (15 cm) wide by 18 inches (45 cm) long on an average

In addition to surface dusting, particularly patch dusting the rodent burrows as well as all other enclosed harbourages (e g, those below double floors, between double walls or above false ceilings) must be thoroughly dusted with the aid of appropriately powerful pumps so as to cover as far as possible the entire inside of these enclosures with a light film As stated in the handbook on rat borne disease, [17] the foot pumps as used for calcium cyanide distribution are satisfactory for treating burrows and enclosures of less than 50 cubic feet (1 4 m^3) in volume Rotary or knapsack dusters ought to be used for larger enclosed or partially enclosed spaces When dealing with rodent holes in vertical surfaces it is well to place a patch of DDT dust within their entrances after pumping has been done in the above described manner

In places where plague is present, and particularly in the case of houses where rat or human plague has become manifest, it may be advisable to apply, in addition to the above described methods, more intensive pro

cedures of DDT dusting Attention ought to be paid in this connexion to the beds and clothes of the people and to the supplies and stores and possibly also to articles of furniture such as easy chairs settees cupboards, and cabinets Care must be taken however, that DDT is not spread on foodstuffs destined for consumption by man or domestic animals or on eating drinking and cooking utensils

Some workers applied DDT dust to the inside of the clothes which were worn by people living in plague affected houses or settlements in the same manner as this insecticide is used for the control of louse borne typhus Ordinarily however, there is no need to use this tedious procedure in plague-control work

It is most desirable to evaluate the results of DDT dusting campaigns by trapping rats in the treated localities and ascertaining the flea indices and/or the percentages of flea infestation of the rodents Particularly reliable results will be obtained if these values can be compared with those ascertained under analogous conditions before DDT dusting had been started Whenever possible such rodent flea surveys should be continued or repeated at suitable e g, monthly intervals, so as to get indications for the necessity of re applying the insecticide

The use of DDT for the purposes of flea control is without danger for man or domestic animals if the following precautions are adopted

1 On account of its similarity to flour supplies of DDT dust must be conspicuously labelled and kept in safe custody

2 Though in contrast to the solutions and the emulsions DDT dust is absorbed by the skin only when this is greasy or oily it should not be allowed to remain on the skin Workers should wash their hands frequently particularly before eating

3 Workers should wear gauze cotton masks when dusting DDT Dust respirators are preferable when powerful pumps are used

4 As noted DDT dust must not be allowed to come in contact with foodstuffs Particularly as stated in this connexion in the handbook on rat borne disease [17] it should not be applied to sacks of flour salt sugar cornmeal or stored grain Grain sweepings from treated establishments should not be used to feed animals (Ludwig & Nicholson [115]) Eating drinking and cooking utensils must also be safeguarded

5 Dogs may be disinfested with DDT dust by placing it on their necks where it cannot be licked off Cats should not be dusted with DDT

If DDT is swallowed vomiting should be induced immediately and the stomach should be washed as soon as possible A saline cathartic should then be administered If tremors or other nervous symptoms appear phenobarbital should be given Intravenous administration of 10% calcium gluconate found effective by Vaz et al [20] in DDT poisoned dogs might be considered

Results

The outstanding value of DDT application in rodent flea control is exemplified by the following observations

Excellent results have been obtained with DDT dusting in the control of *cheopis* borne typhus in the southern parts of the USA Thus Morlan & Hines [144] (see also Hill et al [85]) reported that in two counties, where patch dusting had been done five times during the period of April 1946 to September 1947, effective control of this disease was obtained and maintained without further effort for about three years after completion of the last dusting cycle

In the experience of Macchiavello [119, 120] DDT dusting gave most satisfactory results in the control of various plague manifestations in Peru Thus reporting on the use of this method in the 1945 outbreak at Tumbes, he stated that

> The effectiveness of the application of DDT can be appreciated by (*a*) the stopping of the epidemic 4 days after finishing the first application of DDT (*b*) the 81 6 per cent lowering of the flea infestation of the rats and the 87 9 per cent diminution in the numbers of fleas found in rat nests after the first application of DDT There was a final reduction in the number of fleas in the epizootic foci of over 90 per cent Rat plague was reduced 75 6 per cent after the first application of DDT and 100 per cent after the second

As recently reported by Mercier,[13] systematic use of house dusting in the town of Tananarive, Madagascar, carried out twice yearly with mixtures consisting of equal parts of 5% or 10% DDT and 5% or 10% benzene hexachloride immediately after mixtures of these insecticides had been sprayed for the purposes of malaria control gave most gratifying results Human cases in Tananarive, which had become markedly less frequent since partial use of DDT had been made from 1947 onwards, remained altogether absent after the systematic campaigns had been started in 1949 Whereas previously 6 8 fleas had been found on each trapped rat the infestation rate fell to 0 25 (1 flea per 4 rats) after systematic disinsectization had been started The *cheopis* index was after that time 0 05 as against at least 1 50 previously

Satisfactory results with DDT in the control of plague were also obtained in South Africa Thus, as stated by Davis [38] systematic dusting of some 6 000 huts carried out every 4 months in Ngamiland with 1/2 pound (225 g) of the dust per hut, led to a most marked reduction of the flea incidence After dusting had been done for the 7th time only 5 fleas were found in one of the 500 huts sampled

Wagle & Seal,[227] in a report submitted in 1952 to the WHO Expert Committee on Plague, adduced ample evidence that in India also application of DDT had usually given gratifying results, so that

> Most of the public health workers agree that DDT has been able to keep down plague infection in their States since its introduction on a reasonably large scale and they feel that the results obtained give every promise of a possibility of eradication of plague through its concerted use

The methods of DDT application mainly utilized in India for the purposes of plague control were (a) indoor residual spraying with emulsions containing 5% of the insecticide and/or (b) treatment ("insufflation") of the rat burrows with 10% DDT dust. On the basis of the experiences of Viswanathan & Rao,[1] [6] Wagle & Seal stressed the advisability of combining these two methods. In their opinion it was best to use both procedures simultaneously but if funds were limited, it seemed permissible to apply DDT in the form of sprays during plague outbreaks and to dust the burrows during the interepidemic periods. Though lesser dosages were often used better results were obtained when sufficient amounts of the emulsions were applied to produce deposits of 70-75 mg of pure DDT per square foot (7-7.5 mg/m²).

It is of great importance to note that application of DDT was found to be not only far more effective but also more economical than that of calcium cyanide. Thus in the Nilgiris district of Madras State, India, the cost of the 2 000 pounds (907 kg) of 10% DDT dust needed to treat 90 village units once yearly was Rs 1 750/-. Should calcium cyanide have been used instead, three applications instead of one would have been required and the cost of the 3 000 pounds (1 360 kg) of the material needed for this purpose would have been Rs 4,500/-.[91]

Benzene Hexachloride (BHC)

Among the five known isomers of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane, empirical formula $C_6H_6Cl_6$) the gamma isomer has been found to possess marked insecticidal powers. It is available in the form of a light buff to tan-coloured powder, in the form of solutions made with organic solvents, of emulsions and also of pellets to be used for fumigation. As summarized by Andrews & Simmons,[3] the toxic action of BHC against insects may be as a contact poison, as a stomach poison, as a fumigant, or as a combination of the three. On account of this wider range of action BHC kills insects more rapidly than DDT does but, because it is volatile, its residual action is considerably less long lasting than that of the latter insecticide.

The superior pulicidal effect of DDT was well demonstrated through comparative experiments carried out by Sharif[29] in the Haffkine Institute, Bombay, India. He established that DDT admixed with sand in a ratio of 1/200 000 exerted a residual action for as long as 238 days, whereas BHC if used under the same conditions in a concentration of 1/5 000 retained a residual action for only 48 days. Analogous differences were found in tests conducted by pumping DDT and BHC respectively into artificial rat burrows.

As recorded by Wagle & Seal,[227] field trials in India also showed that the action of BHC on the rat fleas was less marked and persistent than that of DDT

As shown experimentally (Furman,[57] Vashkov & Serebryakova,[19] van Someren [06]), benzene hexachloride could exert a lethal action on rats and mice Van Someren [06] maintained in this connexion that application of BHC dust at the rate of about 2 g (60 mg gamma) on floors, or dusting of rat runs and burrows, or poisoning with baits containing this chemical might be of value for the control of *R rattus, R natalensis*, and—to a lesser extent—mice *Arvicanthus*, which seemed more resistant to BHC than the above species, was thought to be amenable to higher doses particularly if given in bait form

Other Insecticides

It may be claimed that there is no need to enter into a detailed discussion of the formerly used methods of flea control, such as spraying infested premises with kerosene soap emulsions, because these time honoured but little effective procedures have become obsolete since DDT and, to a lesser extent BHC have proved incomparably more efficient However, the question of to what extent other modern insecticides exert a pulicidal action is of importance in so far as their use might come in question in areas where the flea populations have become DDT resistant The following findings have been recorded in this respect

Chlorinated benzenes

As established by Ernst & Meijers,[35] 1 hydroxy symmetric polychlorine benzene as a 5% powder appeared as effective against fleas as 5% DDT preparations In the doses used for the disinfestation of cats and dogs, it was not toxic for these animals

As far as can be judged from limited experiences made by Laurans [110] another chlorinated benzene compound, polychlorocyclane sulfide was effective against dog fleas (*Ctenocephalides canis*) if used as a 4 5% powder

Chlordane

Chlordane like DDT a chlorinated hydrocarbon, with the empirical formula $C_{10}H_6Cl_8$ is available in the form of powders, solutions in oils and emulsions, and stands in regard to its volatility and residual action between DDT and gamma benzene hexachloride Like the latter it exerts a fumigant action besides being apt to kill various insects by contact action It is compatible with DDT (Gray [?])

As stated in a recent report [171] 'a 10% chlordane dust is recommended for controlling soil infestations of cat and dog fleas A single application of this material has given effective control of infestations, whereas repeated applications of DDT would have been required

Similarly it was stated by Kilpatrick & Fay[108] that *Ctenocephalides felis* infestations which could not be effectively dealt with by three applications of 5% DDT dust were much reduced or even eliminated through the use of 5% chlordane. To explore the action of this insecticide on DDT resistant rodent fleas would be of considerable interest.

Organic phosphates

Because as noted before organic phosphates are extremely toxic to man and because they possess very limited residual action (Upholt [18]) they should not be used for the purposes of flea control.

Organic sulfur compounds

As stated by Velbinger,[1] phenothiazine ($C_{12}H_9NS$) when applied in the form of dust or washes to dogs exerted a lethal action on *Ctenocephalides canis*.

According to Frear,[56] phenothiazine which is widely used as an anthelmintic in veterinary medicine has many desirable characteristics as an insecticide but is apt to show a variable action and occasionally causes dermatitis in man.

Organic thiocyanogen compounds known under various proprietary names exert according to Kerr [10] but little action on fleas as compared to that of DDT. As stated by Upholt,[18] these compounds have been used in combination with pyrethrum in order to ensure that insects knocked down by the latter would be killed instead of recovering. Since however the thiocyanates are presumably more toxic than other substances exerting a synergistic action when used with pyrethrum they have to be utilized with caution.

Piperonyl compounds

Andrews & Simmons[3] noted that piperonyl cyclonene because it exerted a synergistic action on pyrethrum appeared to be promising for flea control if used in combination with the latter but lacked the desired residual toxicity for rat fleas in particular.

According to Gray[7] piperonyl butoxide which is also used in combination with pyrethrum had residual properties and because it was free from hazards to warm blooded animals could be freely used around foodstuffs. As noted below Smith [91] found that piperonyl butoxide in combination with pyrethrum extract exerted within 24 hours a toxic action on *X. cheopis* and on *Ctenocephalides felis*.

It should be noted in this connexion that recently attention has been paid in India to the possibility of using the locally available pyrethrum instead of imported synthetic insecticides particularly DDT. Experiments in this direction were carried out in the Haffkine Institute with powders

containing pyrethrins in a concentration of 0 2% on mixed populations of *X cheopis*, *X astia*, and *X brasiliensis* Weekly observations made in order to test the residual effect of this powder showed that, though 100% of the fleas exposed for 15 minutes were knocked down on the first day, the knock down effect was only 75% after seven days, 50% after two weeks, and nil after four weeks [9]

One must fear that under natural conditions the residual effect of such powders would be still less satisfactory

Sodium fluoride

Sodium fluoride (NaF) known as an insecticide for more than fifty years was recommended in 1940 by Roubaud [18] for flea control in houses Roubaud found that, while the action of this compound on adult fleas was too slow to make it useful for disinfesting animals such as dogs it exerted a larvicidal action, killing for instance in a concentration of 10% *Ctenocephalides canis* larvae in 18 24 hours An *X cheopis* population kept in a glass jar was found to be annihilated a few days after small doses of NaF had been applied Premises infested with dog or cat fleas could be freed from these parasites by spreading sodium fluoride either in pure form or in a concentration of 50% on the floors at the rate of 2 g per square metre The price of this chemical was low and its toxicity for man and domestic animals was 30 times less than that of arsenites In Roubaud's opinion sodium fluoride was apt to be useful for combating loose *X cheopis* living in grain debris

It might be interesting to establish whether or not NaF could be used together with pyrethrum

Comparative tests

Smith,[204] comparing the action exerted within 24 hours by 619 insecticides on *Ctenocephalides felis* and *X cheopis* with that of DDT, found that at a concentration of 0 5% 29 of these compounds proved more effective than DDT, 24 were within the range of effectiveness of DDT and 566 were less effective than DDT He stated in particular that

> The outstanding toxicants were heptachlor dieldrin (compound 497) aldrin (compound 118) and benzene hexachloride (95 per cent gamma isomer) Chlordane and parathion were more effective than DDT at concentrations of 0 5 and 0 05% and toxaphene and pyrethrum extract plus piperonyl butoxide at 0 5% only Methoxychlor and other analogs were about equal to DDT

Interesting as these findings are they are of only limited practical value because (*a*) the residual action of the compounds found to compare favourably with DDT or to be as effective as the latter insecticide was not determined, and (*b*) the DDT concentrations used in these comparative tests (0 5%, 0 05% and 0 005%) were far below those applied in actual practice

Fumigants

The comparative value of various fumigants for the destruction of rodent fleas is well illustrated by the observations of Stewart & Mackie [209] whose principal findings are embodied in table XXIII

Though as shown in the table hydrocyanic acid gas gave by far the best results Stewart & Mackie [209] stressed that in actual practice the experiences made with the application of calcium cyanide were not fully satisfactory in the case of the wild rodent fleas or in that of the rat fleas The two workers advocated therefore the use of methyl bromide which if applied to each opening of ground squirrel burrow systems in quantities of 10 ml was found to be fully effective against the fleas as well as against the rodents There can be no doubt, however that nowadays it would be both more expedient and far more safe to use DDT instead of methyl bromide for the purposes of flea control

However fumigation procedures and still more application of calcium cyanide continue to be of importance for the disinfestation of grain consignments under the conditions usually prevailing in plague affected localities —the more so as it was established through the observations of Pandit et al [1 7] and of George & Webster [6] that exposure to the sun or to hot air as formerly often done was unreliable for the disinfestation of grain bags

TABLE XXIII RELATIVE EFFICACY OF VARIOUS FUMIGANTS AGAINST ADULT FLEAS (DIAMANUS MONTANUS AND HOPLOPSYLLUS ANOMALUS)

Fumigant [a]	Temperature		Volume of gas [b] (ml)	Time of exposure (hours)
	(F)	(C)		
Carbon disulfide	71	21 6		1
Chloropicrin	77	25 0	5	1
Ethylene oxide	75-80	3 9 [illegible] 6	2 10	0 5-4
7 % ethylene oxide + ethylene dichloride	78 8[illegible]	[illegible] 6 27 8	10	1
HCN	71 87	21 6 30 6	0 1-0 5	0 1[illegible] 5
Methyl bromide	72 78	[illegible] 2 [illegible] 6	0 10	0 16[illegible] 1
Propylene oxide	78 85	[illegible] 29 5	10	1 [illegible]
Sulfur dioxide	76	4	10	1

Only 100 kill recorded

[a] Some other fumigants including carbon monoxide and pure ethylene dichloride were found to exert no lethal action on fleas

[b] The tests were made in a glass fumigation chamber with a capacity of 1[illegible] ml

The following experiment made by George & Webster is particularly instructive

"On one occasion in Cumbum village a bandicoot was found dead of plague in a grain store The fleas on the carcass ten in number were removed About 700 bags of grain

were removed and placed singly on a cement platform exposed to bright sunlight throughout a whole day in November 1932 The maximum shade temperature that day was 82°F [28°C] At night twelve de flead guinea pigs were allowed to wander loose among the bags Next day eleven of these [animals] were caught and searched for fleas Thirty six *cheopis* and *astia* fleas were recovered

Application of hot air, used on dull days to disinfest grain in bags, was equally unsatisfactory because, as stated by George & Webster,[6] it was not possible in this way to maintain a temperature of 120°F (49°C) for 45 minutes as required for the destruction of fleas

Further observations in the Cumbum Valley, Madras State, showed, however, that satisfactory results could be obtained by applying calcium cyanide (*a*) to grain bags put in fumigation chambers or (*b*) to the bags loaded on bullock carts which were covered for this purpose with oiled tarpaulins, in sheds erected on the roadside[60 61 93] It was found that the following HCN concentrations and minimum periods of exposure were required to de flea the various commodities usually encountered in these wayside plague control stations

Articles	*Quantities of HCN* required per 1 000 cubic feet***	
	*in airtight chambers****	*under oiled tarpaulins*
Personal effects	1 oz for ½ to 1 hour	1 oz for 1 hour
Rice paddy lentils coriander etc	3 oz for 3 hours	4 oz for 3 hours
Bran coffee husks sesamum seeds etc	4 oz for 8 hours	5 oz for 8 hours
Cotton and flour	12 oz for 5 hours	16 oz for 8 hours

* Obtained through application of approximately 4 ounces of calcium cyanide dust

** 1 ounce per 1 000 cubic feet = 1 g/m³

*** Capacity of about 3 000 cubic feet Enclosed on three sides and on top by solid masonry work Entrance tightly closed with the aid of an oiled tarpaulin Calcium cyanide was pumped in through lead pipes in walls

Remarks (*a*) To test the efficacy of these procedures observations were made in 1935 with live fleas in muslin bags left at various levels inside the grain bags and outside them

(*b*) Penetration of the gas was facilitated if the bags were not too tightly packed

The use of methyl bromide for the purpose of freeing grain cargoes from plague infected fleas was recommended by Stewart & Mackie[09] It must be noted however, that recently van Tiel[15] considered further tests necessary to exclude the possibility of an absorption of methyl bromide into grain He considered it preferable, therefore, to fumigate grain with methallyl chloride (β methylallyl chloride or 3 chloro 2 methylpropene) which is safer to handle in so far as it has a repulsive odour However this compound is inflammable and at certain concentrations explosive when admixed with air Moreover, though found to be effective against bed bugs and lice its action on fleas has apparently not been explored

Bouhelier & Foury[18] recommended trichloroethylene ($CHCl$ CCl_2) for grain disinfestation Experimentally, this volatile, non inflammable fluid which is used as an anaesthetic in clinical medicine, killed *Ct canis* in 19½ hours if used at the rate of 100 ml per cubic metre or 1 fluid ounce per 10 cubic feet at temperatures ranging from 20° 25°C (68° 77°F)

It would not seem that application of the above fumigants is more advantageous than that of calcium cyanide for the purpose of freeing grain supplies from fleas under field conditions Hence until further research leads to the introduction of improved procedures calcium cyanide applica tion may be considered as the method of choice in dealing with actually or potentially flea infested grain consignments under the conditions usually prevailing in and near rural plague areas

DIRECT CONTROL OF BUBONIC (ZOOTIC) PLAGUE

Having dealt in the preceding pages with the methods of indirectly controlling plague through steps taken against the reservoir rodents and the insect vectors of the infection, it is now necessary to review the measures by which man the victim of the infection may be safeguarded For obvious reasons separate consideration must be given in this respect to the bubonic (zootic) and to the pneumonic (demic) type of the disease

Hospitalization of Patients

It may be maintained that from the viewpoint of plague prevention it is immaterial whether or not patients suffering from bubonic plague not complicated by lung manifestations are isolated Such sufferers are not directly contagious and as proved by the usual absence of instances of secondary infection in their households the danger of an indirect conveyance of *P pestis* from them to those near them through insect vectors is as a rule inconsiderable Even if the sufferers left in their houses should sub sequently develop secondary lung involvement the danger of a spread of pneumonic plague can now be effectively averted through abortive treatment of their contacts

If however, one disregards the somewhat arbitrary distinction between preventive and curative medicine which are united in the endeavour of benefiting mankind hospitalization of bubonic (zootic) plague patients is of great importance in view of the far superior facilities available in the wards not only for efficiently treating but for adequately nursing the sufferers

Hence while preferably avoiding forceful means plague workers should use every possible inducement to arrange that the patients are quickly hospitalized However in places remote from hospitals or possess ing no adequate means of communication one ought to weigh the advan tages of hospitalization against the risks to which a long and/or inade quately conducted transport exposes the patients One should also keep in mind that among groups of people unfamiliar with or prejudiced against

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It may be maintained that from the viewpoint of plague prevention it is immaterial whether or not patients suffering from bubonic plague not complicated by lung manifestations are isolated. Such sufferers are not directly contagious and, as proved by the usual absence of instances of secondary infection in their households, the danger of an indirect conveyance of *P. pestis* from them to those near them through insect vectors is, as a rule, inconsiderable. Even if the sufferers left in their houses should subsequently develop secondary lung involvement, the danger of a spread of pneumonic plague can now be effectively averted through abortive treatment of their contacts.

If, however, one disregards the somewhat arbitrary distinction between preventive and curative medicine, which are united in the endeavour of benefiting mankind, hospitalization of bubonic (zootic) plague patients is of great importance in view of the far superior facilities available in the wards, not only for efficiently treating but for adequately nursing the sufferers.

Hence, while preferably avoiding forceful means, plague workers should use every possible inducement to arrange that the patients are quickly hospitalized. However, in places remote from hospitals or possessing no adequate means of communication one ought to weigh the advantages of hospitalization against the risks to which a long and/or inadequately conducted transport exposes the patients. One should also keep in mind that among groups of people unfamiliar with, or prejudiced against

modern medicine, insistence on hospitalization, while beneficial to the affected individuals, may greatly enhance the difficulties of obtaining the goodwill and co operation of the people It is important to note in this connexion, that some workers, such as Mathur & Goyal,[131] Shamanna & Hedge,[193] Shamanna & Kalappa,[191] and Simeons & Chhatre [0] in India, and also the present writer in China, were able to obtain satisfactory results when treating bubonic plague patients in their homes

If no permanent plague hospitals or wards are available, care must be taken to house the patients in buildings which are, or can be rendered, free from rats A special ward, preferably in a separate building or in a shed or tent erected for this purpose should be provided for, where the newly arrived patients are undressed, bathed or washed if necessary freed from vermin, and dressed in hospital garments

The clothes in which the patients come must be disinfested in steam sterilizers or delousers, or—if such permanent facilities are unavailable—with the aid of calcium cyanide or a fumigant in an airtight chamber or container Application of DDT dust followed by storage of the clothes would also be satisfactory

The staff handling the newly admitted patients or their clothes should be provided with flea proof garments Such garments should also be worn by the staff members attending the patients in the wards in cases where no hospital garments can be issued If so, it might be advisable to apply DDT dust to the persons and clothes of the patients as practised for the pur poses of louse borne typhus control

The medical officers and nurses attending plague patients in the wards should preferably wear masks even if the sufferers show no manifest signs of lung involvement

Management of Contacts

Though it was often considered necessary to isolate the contacts of bubonic plague patients for six days or even longer in quarantine camps it may be asserted that, in view of what has been stated in the foregoing section there was never any real need to insist upon this most unpopular practice as long as the patients had been hospitalized or, if left in their houses, remained free from lung complications Nowadays when DDT is available to render the affected houses safe, the method of confining the contacts of patients suffering from bubonic plague without lung involvement in camps is altogether obsolete

Evacuation

Voluntary removal of the people from places where plague had appeared or had become rampant, is no doubt the oldest method used to prevent the

spread of this disease For instance it is definitely known that this procedure had been adopted for many centuries in northern India where the people left their homes as soon as the rats began to die (Patel [160])

However, while one must admit the soundness of the principle of removing the people from places where hordes of plague infected fleas lurk several reasons militate against its implementation Short of the barbaric practice sometimes adopted formerly of burning down plague infected houses or even settlements enforced evacuation is the most unpopular of all anti plague measures To make adequate arrangements for housing and feeding the evacuated is difficult Moreover unless proper methods of disinfestation are applied the evacuated may bring along infected fleas in their effects or even on their persons so that the spread of plague among them is not cut short

While for these reasons the advisability of a mass evacuation of plague infected settlements or precincts was questionable in the past adoption of this method has now become superfluous because instead of removing the people from the flea vectors the latter may be effectively dealt with in loco

At the same time however consideration ought to be given to the advisability of temporarily removing the inhabitants of individual houses or groups of houses which on account of a particularly heavy rat infestation have become hot beds of the infection so as to get elbow room and sufficient time to deal adequately with the rodent harbourages

Mass Vaccination

It is not surprising to find that in the field as well as in the laboratory the results obtained with plague vaccination by different workers and consequently the opinions they formed regarding the value of this prophylactic method varied most markedly The potency of the vaccines they used was bound to be markedly different The same holds true of the dosage used—a factor of particularly great importance in the case of the killed vaccines which instead of being given in two doses at an interval of 5–7 days as is considered essential in principle were actually most often administered once only More important still the statistical evidence presented in regard to the efficacy of plague vaccination though ample is often not satisfactory mainly because the groups of the vaccinated and unvaccinated were not under the same risk of infection (see Otten [153])

Nevertheless though it is not possible to accept the exaggerated claims made by some advocates of plague vaccination there is no reason to revise the opinion reached after a careful consideration of the evidence by competent reviewers such as the Indian Plague Commission (quoted by Simpson [203]) Dieudonne & Otto [44] and recently Meyer [137] that the method is of definite value in the prevention of plague It is important to note that Meyer was

able to base this conclusion not only upon reliable statistics, such as those of Otten (1936, 1940 1941)[153 155] Girard & Robic (1936, 1938)[88 87] Girard (1946),[64] and Patel & Rebello (1948),[161] but also upon observations on the appearance of immune bodies in volunteers to whom plague vaccines had been administered (Meyer & Foster[138] Meyer 1948, 1953[136 137])

Being due to a process of active immunization, the protection conferred through plague vaccination reaches an effective degree only after a period usually assumed to last 5-7 days after administration of the vaccine It is inevitable, therefore, that persons who incubate the infection at the time of vaccination or who become infected soon afterwards, develop plague even though they have been vaccinated It was undoubtedly due to such occurrences, which are bound to be particularly frequent if plague is rampant, that soon after Haffkine started to administer his vaccine claims were made as to the existence of a negative phase following vaccination, during which the vaccinated were supposed to be more susceptible to the infection than the unvaccinated As noted by Stevenson & Kapadia,[208] in a discourse delivered in 1899 before the Royal Society, London, Haffkine definitely denied the existence of such a negative phase

Calmette & Salimbeni,[9] on the contrary, noted during the 1899 out break at Oporto that animals during the period of immunization with heated cultures were extremely sensitive to very small doses of the virus doses which were rarely mortal to non vaccinated animals' (summary by Calmette, quoted by Stevenson & Kapadia[08]) Since, however, Calmette & Salimbeni experimented with a few animals only and used a vaccine which, because it had been heat killed at a temperature of 70°C (158°F) could have possessed but little immunizing value not much credence can be given to their findings

Nevertheless, during the years following the introduction of Haffkine's vaccine there was so much fear of a negative phase in Bombay that persons living in plague houses were given only half the vaccine doses used for people who apparently had been under no risk of infection This practice was continued until 1905 when Liston,[115] repeating Calmette & Salimbeni's experiments with a small number of guinea pigs found no evidence of the existence of a negative phase Liston's findings were confirmed through ampler tests carried out by Stevenson & Kapadia[08] which showed

(1) that there is no negative phase or period of increased susceptibility of rats to plague after the administration of anti plague vaccine even within one and a half hours after the operation,
(2) that the production of immunity among rats commences within a few hours of inoculation and increases in amount till the 2nd or 3rd day after anti plague vaccination"

More important still, observations confirming the experimental findings of Stevenson & Kapadia have been made in the course of vaccination campaigns Bannerman (quoted by Dieudonne & Otto[44]) maintained in this connexion with much reason, that if the susceptibility to plague were

increased immediately after vaccination, as was claimed by Calmette & Salimbeni there ought to be a higher plague mortality among the recently vaccinated than among the unvaccinated Actually however, the mortality among 358 persons developing plague within 10 days after vaccination including 43 who were already ill when vaccinated or fell ill on the same day, was 48% as against a death rate of 73 7% in a control group of 5 079 unvaccinated *In a smaller group observed at Dharwar, only 36 5% of* 74 persons developing plague within 10 days after vaccination succumbed as against 90 8% deaths in the control group

Patel & Rebello[161] recently recording the results of a mass inoculation campaign with Haffkine Institute vaccine also found that there was no adverse negative effect observed among those inoculated after exposure to infection and hence it would seem unwise to refuse inoculation to contacts from fear of negative phase effect

Summarizing the experiences made in China with agar grown killed vaccines Pollitzer[166] likewise stated

"We never found any evidence of a negative phase—on the contrary when administering for curative purposes during the 1928 South Manchurian outbreak single vaccine doses to recently admitted bubonic plague patients we saw that the life of these sufferers was often prolonged"

That administration of live avirulent vaccines does not produce a negative phase is well shown by the observations of Grasset[0] who found only 15 cases of plague among 24 000 immunized persons within 10 days after vaccination

Hence though statements to the contrary have been made by a few recent observers either on experimental grounds (Pons & Advier[171]) or on account of field observations (see Simeons & Chhatre for example[01 0]) it may be asserted that plague vaccination is not followed by a negative phase during which the vaccinated are temporarily more susceptible to the infection Since however administration of plague vaccines does not prevent development of the disease in persons who were incubating the disease or who became infected during the days immediately following vaccination it is obviously preferable to use this preventive method during the interepidemic period and not when plague particularly human plague has already become manifest

As has been discussed in chapter 3 no doubt can exist that under adequate conditions equally good laboratory results can be obtained with killed and live avirulent vaccines respectively

It is gratifying to note that an analogous conclusion has been reached in regard to human plague vaccination by Meyer[13] who stated that

"Any plague vaccine whether killed or living provided it either inherently contains 2 to 3 mg of Fraction I or is capable of producing this amount when inoculated into the

body of man will favourably alter the susceptibility of 50 per cent of the inoculated This *basic immunity may even improve after the first month and may persist for at least* 6 months'

Meyer emphasized, however, the great importance of revaccination concluding that

Annual revaccination progressively improves the immune status of the population groups and this creates the impressive results reported from countries in which this principle has been adopted as an aid in the control of plague in endemic regions

In view of the statements made above the choice of the vaccines to be used for the purpose of plague prevention depends upon practical considerations rather than upon fundamental differences between the products The casein hydrolysate vaccine now manufactured in the Haffkine Institute is certainly an excellent, exactly standardized product but shares with all killed vaccines the drawback that in order to be fully effective, it must be administered in two doses given at an interval of 5–7 days—a procedure which as a rule is rather difficult to adopt Live vaccines are fully effective in single doses but thus far have to be used soon after manufacture, a procedure not easy to adopt when it is necessary to carry out campaigns in remote areas However, it deserves great attention that in contrast to previous experiences (see chapter 3) recent preliminary tests with a lyophilized EV vaccine prepared by Girard have given promising results in vitro and experimentally[4] Still even if such lyophilized products should be found suitable for human vaccination, it might remain difficult to use them in localities distant from adequately equipped laboratories

The dosages for the administration of the casein hydrolysate vaccine now produced in the Haffkine Institute, Bombay, are as follows (Greval[73])

Age and sex		*First dose (ml)*	*Second dose (in 5 7 days) (ml)*	*Annual revaccination (ml)*
Men		1 0	1 5	1 5
Women		0 75	1 0	1 0
Children	1-4 years	0 2	0 3	0 3
	5 10 years	0 3	0 5	0 5
	11 15 years	0 4	0 75	0 75

Note It should be kept in mind that these dosages particularly those for women have been worked out according to Indian standards

Agar-grown killed vaccines are usually administered in an initial dose of 0 5 ml, followed 5–7 days later by administration of 1 ml The latter dose (1 ml) is also adopted for the purposes of revaccination Lesser doses must be given to adults of low body weight and to children in proportions analogous to those indicated in the case of casein hydrolysate vaccine

Live avirulent vaccines are administered once in an adult dose of 1 ml (Girard & Robic[66] Grasset[0]) The proportionally lower doses recom

mended by Grasset [0] for children were 0 5 ml for the age group of 5 to 12 years and 0 25 ml for those under 5 years

If due regard is given to the principles of asepsis the local and general reactions produced by the now available plague vaccines are as a rule mild or at most moderate The conclusion arrived at in this connexion by the WHO Expert Committee on Plague [37] at its second session was that

"With living vaccines local reactions are essential while with killed vaccines satisfactory protection can be obtained with preparations producing neither local nor general reactions"

Serum Prophylaxis

As summarized by Dieudonne & Otto [41] some early workers reported favourable results obtained through mass prophylaxis with plague immune serum However Dieudonne & Otto pointed out that the validity of some of these observations was doubtful and stressed moreover that the method was disadvantageous in view of the short duration of the protection afforded and the high price of the serum which would render it impossible to repeat the injections even if there were no danger of anaphylaxis [e] Dieudonne & Otto maintained however that it would be advantageous to combine the prophylactic administration of plague immune serum with that of plague vaccine so as to obtain a rapidly effective as well as a longer lasting protection They noted that the use of this combined method had given favourable results during an outbreak in Kobe Japan

Though good success with this combined method was also recorded by some recent workers the serum doses they used were often so small (5 ml or even less) that it is difficult to consider them as effective No doubt more reliable results could be obtained with higher doses but the cost of using these during large scale campaigns would be prohibitive Moreover as shown by an observation of Mackay Dick [11] even plague serum doses of 5 ml may produce severe urticarial reactions

It would appear therefore that the method of combined plague serum and vaccine prophylaxis has little if anything to recommend it As already stated vaccination campaigns should be conducted before plague becomes manifest so as to avoid the appearance of the disease in persons already incubating the infection when they are vaccinated Should it be necessary to vaccinate during a plague epidemic application of DDT would be far more effective in preventing infection of the not yet immunized than combined serum administration One may claim therefore that nowadays this combined method of prophylaxis is of historical rather than of actual interest

[e] That the second administration of prophylactic serum doses after an injudiciously long interval is rather dangerous was proved by an observation made by Lloyd during the 1908 outbreak at Guayaquil Ecuador He stated that then a number of private practitioners tried to immunize their clients against plague by injection of two small doses (usually 5 cm) of antiplague serum at an interval of 15 20 days A great number of severe anaphylactic reactions resulted Several of the patients were confined to the bed for days or even weeks but I have not learnt that any death from anaphylaxis resulted

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Comparative Value of the Measures of Control

The comparative value of the various measures against bubonic (zootic) plague enumerated below has been and still is, the subject of considerable debate

Measures to be used permanently
Rodent control intelligence work public health propaganda and education

Measures to be used periodically or temporarily
Application of insecticides, rodent destruction vaccination quarantine measures

General agreement exists that, in order to deal effectively with bubonic plague a proper system of detecting manifestations of the infection particularly of incipient epizootics, is indispensable The great value of obtaining the goodwill and co operation of the people through propaganda and health education is also generally realized The necessity for measures to prevent the spread of plague at distance is likewise universally recognized

However even though the causative role played by the rodents is fully realized the advisability, or even the necessity, of making direct campaigns against these animals the cornerstone of plague preventive work is not rarely doubted In the past, it was at one time believed that vaccination alone would suppress plague (Haffkine, quoted by Dieudonne & Otto [41]) While the fallacy of this hope was soon realized, a number of workers were of the opinion that anti rat campaigns were of no use for the control of plague because no really effective methods were available to combat these animals Now that this impasse has been overcome it is sometimes stated that in view of the success obtainable in plague control with DDT alone other measures, including rat control are superfluous

In evaluating this claim one must admit that

(*a*) as long as the rodent fleas have not become resistant to DDT, single applications of this insecticide, if adequately made form a practically infallible means of preventing or suppressing plague manifestations in man

(*b*) DDT campaigns if repeated at proper intervals, continue to keep the rodent flea incidence at low levels and this low flea incidence is apt to persist for considerably long periods even after the periodic use of the insecticide has been stopped

However whether this prolonged reduction of the flea rate will lead to a disappearance of rodent plague as is sometimes claimed, remains to be seen An observation made by Hill et al [85] in murine typhus control work deserves great attention in this respect As found by these workers, repeated DDT dusting led to suppressions of human typhus incidence prevalence of complement fixing antibodies and rat flea abundance which persisted for over two years following the last dusting campaigns in September 1947 They added, however that

A moderate rise in prevalence of murine typhus complement fixing antibodies in the domestic rat reservoirs in the two treated counties in 1949 accompanied by a moderate increase in abundance of rat fleas suggests the necessity for further surveillance

These observations tend to confirm the opinion held by the majority of plague workers and fully shared by the present writer that DDT application though of great value in plague control work does not obviate the necessity of rodent control work

As stressed before exclusion methods should be used as far as possible for the latter purpose in preference to eradicative work The WHO Expert Committee on Plague (1952) [37] while admitting that it was impossible to recommend one rigid scheme of plague control endorsed this view by stating that under all circumstances the ideal method to fight plague was to cut short the contact between rodent and man through house improvement

A further much debated question is whether the availability of DDT renders plague vaccination superfluous There is no doubt that DDT application is the method of choice during plague outbreaks when it is preferable not to resort to vaccination so as to avoid the development of the disease in persons who are already infected when they receive the vaccine or who contract the infection soon afterwards because they have not yet become immunized

At the same time however it would be unwise to discard the use of vaccination applied during the interepidemic periods and followed by yearly revaccination during those periods for the following reasons

(a) As stated in chapter 8 vaccination considerably improves the chances of curing plague patients—a factor of particular importance in localities where no or limited possibilities for the use of antibiotics are available

(b) In vast and sparsely populated rural plague areas where it may be difficult to visit every house for the purpose of DDT administration it may prove easier to vaccinate the people assembled for this purpose at suitable points or to vaccinate those assembled for other purposes e g at markets or fairs

Vaccination might also serve as a sheet anchor in plague infected localities where the fleas have become DDT resistant

CONTROL OF PNEUMONIC (DEMIC) PLAGUE

In contrast to the control of the insect borne zootic form the prevention of pneumonic plague because it depends almost exclusively upon the protection of man from man is quite easy to accomplish The principal methods to be adopted when dealing with patients suffering from primary

pneumonic plague as well as with those developing secondary lung involvement in the course of a primarily bubonic attack will now be discussed

Case Detection

The rapid suppression of pneumonic plague manifestations depends primarily upon a system of complete, as well as rapid, case detection Every effort must be made, therefore, to detect the presence of the disease in either the secondary or the primary form at the first possible moment so as not to delay the measures necessary for dealing adequately with the situation A prompt diagnosis of primary pneumonic plague is all the more important because as emphasized in chapter 8, the patients are non infective during the first 20 24 hours of illness

The detection of cases during a pneumonic plague outbreak is greatly facilitated if the help of the people can be obtained in this respect Hence instead of trying to hide the occurrence of the disease all available means of propaganda should be used to inform the people of its presence, to acquaint them with its symptoms and signs and to urge them to report all suspicious cases at once, so that the sufferers may be properly cared for and their contacts may be protected

Since, however it is impossible fully to rely upon the help of the people in this respect, a system of house to house inspection must be adopted in communities, precincts, or smaller foci where pneumonic plague shows a tendency to spread Preferably the houses should be visited twice daily, as early as feasible in the morning and as late as possible in the afternoon or evening All inmates of the houses should be seen, and their pulses as well as their temperatures if possible, should be taken Any person showing an increased temperature and/or an unusually rapid or otherwise abnormal pulse, or who appears otherwise to be ill, should be considered as plague suspect, unless he obviously suffers from another disease and should be kept under intensive observation or sent at once to the suspect ward of the hospital

Isolation and Treatment of Patients

Prompt hospitalization of pneumonic plague patients must be insisted upon, both in order to exclude the possibility of a spread of the infection to their families or visitors and to give them optimal facilities for treatment In places remote from hospitals it may be inevitable to make exceptions to this rule but even though it may be possible safely to isolate the patients in their houses or in outhouses or other buildings the chances of effectively treating them at home are poor

Both in order to initiate treatment at the earliest possible moment and to reduce chances for a spread of the infection from the patients during their

transport to the hospital it is desirable to administer to them a dose of a suitable antibiotic before they leave their homes Seal[190] recommended the use of 1 g of streptomycin for this purpose

Besides wards for the accommodation of the patients in the manifest stage of pneumonic plague admission wards as well as sufficiently spacious wards for suspects must be provided for

As in the case of bubonic plague hospitals the admission ward should be installed in a separate building or in a shed or tent erected for this purpose The incoming patients must be undressed cleaned if necessary also dis insected provided with hospital garments and then sent to their proper wards Contact between suspects and those suffering from manifest pneu monic plague must be strictly avoided

It would be ideal to accommodate the suspects in individual rooms or cubicles but this is as a rule impossible However a fair amount of pro tection can easily be afforded to the suspects by placing screens between the individual beds which should not be placed close to one another

Management of Contacts

It is no doubt desirable to accommodate the contacts of pneumonic patients for a period of 6 10 days in quarantine camps instead of leaving them at home—both in order to ensure that abortive treatment is effectively carried out and to detect signs of incipient illness at once However since removal of the contacts to a camp may be so much resented by the people as to lead to the hiding of cases the plague staff should not go too far in insisting upon this measure provided that it is possible to make adequate arrangements for observation and abortive treatment of the contacts in the houses In particular it may be advisable to leave one or two inmates in farmhouses to care for the livestock

Abortive treatment should be started even before the contacts leave their houses In the camp they must be seen at least twice daily when their temperatures and pulses must be taken Those appearing to be even slightly ill must be separated from the rest closely observed and sent to the suspect wards unless they rapidly improve or obviously suffer from another disease Development of manifest pneumonic plague in a camp is a sign of bad management

Protection of Staff

As shown by the deplorably large number of pneumonic plague infections among isolation hospital workers or other staff members, proper precau tions must be taken by all persons attending sufferers from this form of the disease The most important of these precautions is the wearing of a proper mask

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As described by Chun,[79] the mask used in the Manchurian outbreaks

"consists of two layers of gauze enclosing a flat oblong piece of absorbent cotton. It can be easily made by cutting the usual surgical gauze (9 inches wide [23 cm]) into strips each measuring 3 feet [1 m] in length. Each strip is then folded lengthwise so as to contain in the middle a piece of cotton wool measuring 4 inches by 6 inches [10 cm by 15 cm] in area and ½ inch [1.3 cm] in thickness. At either end of the gauze two cuts, each measuring 15 inches [38 cm] are made, thus turning the pad into a two-tail gauze bandage with the central piece of wool over the nose and mouth. The upper tails of each side should be passed round the head above the ear and tied together behind. The lower tails should in a similar manner be passed under the ear and tied.

In individuals with prominent noses a lacuna is left on each side when the mask is adjusted. In order to obliterate the leak, the insertion of small cotton plugs within the upper margin of the mask to fill up the empty spaces on either side of the nose is to be recommended."

As a further precaution, the hospital staff wore a hood made of cloth with a layer of silk 6 inches (15 cm) wide sewn in front of the nose and mouth area. This hood, which had apertures for the eyes, was tucked inside the overall at the neck of the wearer. Goggles were worn, but alternatively another kind of hood provided with a frontal mica window was worn over the gauze cotton mask.[f]

In addition to the masks and, if necessary, hoods and goggles, the workers were protected by hospital gowns closing at the back, and one-piece linen trousers and stockings, as well as by rubber or high leather boots. Rubber gloves were worn when there was danger of coming in contact with sputum. The working apparel, particularly the masks, had to be worn not only in the wards but whenever there was a possibility of coming in touch with plague patients with lung involvement.

Disinfection of Contaminated Objects

While it seems unnecessary to carry out wholesale disinfection of the rooms or houses from which pneumonic plague patients have been removed, careful sterilization of all objects contaminated with their sputum is essential.

Removable articles of little value, which have become soiled with sputum, are best burnt. Garments and bedding, which have become contaminated, ought to be sterilized by steam or boiled. Water at boiling temperature should be poured over sputum deposited on bedsteads, floors, or walls. Deposits on level surfaces should then be covered with lime powder. Contaminated parts of the walls, or preferably the whole walls, should be freely treated with whitewash.

The patients should be given solid and large sputum cups or spittoons which should be frequently sterilized by boiling them together with their contents.

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[f] It is interesting to note that, according to Rooks et al.,[1] repeated laundering of the gauze material used for hospital masks greatly increased the bacterial filtering efficiency of the latter.

The urine and the stools of the patients should be mixed with generous amounts of milk of lime before they are disposed of. Boiling water ought then to be used to clean the containers unless they can be sterilized by steam.

The methods of dealing with the dead bodies of pneumonic plague victims will be discussed later (see page 610).

Value of Vaccination

Killed vaccines

The problem of to what extent administration of killed vaccines was of value for the prevention of pneumonic plague was thoroughly discussed during the International Plague Conference held in 1911 at Mukden, Manchuria.[9] Summarizing these discussions Stanley[197] stated that

"From the evidence available it was not possible to draw definite conclusions as to the value of protective inoculation against plague pneumonia, but the general opinion was that the infection was too massive when the lungs were involved for the ordinary methods in use to be of much value."

As far as the present writer is aware, no further facts have been recorded to establish that administration of killed vaccines is of direct benefit in pneumonic plague epidemics. The method was not used during the 1917-18 Shansi outbreak, Young[241] postulating that vaccination would have given a false sense of security which might have led to a neglect of essential precautions, especially the wearing of masks.

During the 1920-1 pneumonic epidemic in and near Manchuria vaccination was used only in the Russian Coast Province where the disease caused but little havoc. How far it was effective it is impossible to say because (*a*) probably most of the 6,000 vaccinated Russians who remained free from plague were under no real risk of infection and (*b*) nothing is known regarding the fate of the 3,000 vaccinated Chinese.[9]

It is significant that Wu,[238] discussing the management of pneumonic plague epidemics in general, made no reference to the use of vaccines.

Live vaccines

In the opinion of Grasset[70] the efficacy of live plague vaccine in the prevention of pneumonic plague was "shown by the small number of plague cases among immunized persons even among very close contacts of pneumonic cases". It is important to note, however, that most of the latter contacts received prophylactic serum doses as well as vaccine. Thus an old woman who had remained healthy even though she had attended four pneumonic plague victims in her household had been given in addition to live vaccine not less than 100 ml of plague immune serum prophylactically.

Compiling the statistics on a large scale trial of his Tjiwidej vaccine Otten [153] omitted to consider the 84 pneumonic plague patients, 36 of whom had been vaccinated, on the presumption based upon data available from literature and personal research, that this vaccination could not be expected to guarantee any protection against aerogenic infection, at least when subcutaneously administered at the relatively small dose adhered to till now '

Girard,[65] discussing the potential value of EV vaccine in the prevention of pneumonic plague made the following statement

> Our answer to this often asked question is that guinea pigs are effectively protected against the bronchopneumonia which is invariably caused in the controls through intra tracheal plague infection And despite the actual failure of this as well as of other modes of vaccination, it is undeniable that protection against bubonic plague leads ipso facto to a reduction of the pulmonary form which originates from the former type [g]

With this conclusion one must thoroughly agree Though evidently of no direct value in warding off pneumonic plague infection in man plague vaccination is of indirect value by reducing the frequency and/or severity of bubonic manifestations

DISPOSAL OF THE DEAD

In the past, it was often considered necessary to cremate the dead bodies of plague victims, but except that it formed an easy means of disposing of numerous corpses, there was never any real need to resort to this practice which was often utterly repulsive to the people Now, when one may expect that, owing to the availability of effective preventive and curative methods there will no longer be any mass mortality from plague, it is certainly legitimate to bury the dead bodies instead of cremating them, unless cremation is compatible with the religious tenets or customs of the populations concerned

As far as possible the dead bodies of plague victims ought not to be handled and encoffined by their relatives or friends but by burial squads The members of these should be provided with masks protective garments and boots, as well as with thick rubber gloves or stout canvas mittens

It was formerly often recommended that the shrouds or clothes of the dead be moistened with strong antiseptics or that the dead bodies be covered with lime Since, however, such practices, because apt to counteract putrefaction, may prolong persistence of the causative organisms in the dead bodies, they are not to be recommended

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g " A cette question maintes fois posée nous répondrons que le cobaye est effectivement protégé contre la bronchopneumonie que détermine invariablement chez les témoins l inoculation de virus dans la trachée Et ce qui est indiscutable malgré les échecs à l actif de cette vaccination comme de toute autre c est que la protection contre la peste bubonique entraîne *ipso facto* la réduction des formes pulmonaires *qui en dérivent*

It may be well however, to place a layer of lime or another absorbent material into the coffins before the dead bodies are put in

Burial in graves sufficiently deep to prevent access of rodents or other rapacious animals is essential

The garments and other belongings of bubonic plague victims dying in their houses must be de flea d unless ample previous use of DDT has been made In the case of pneumonic plague victims due attention must be paid to the sterilization of all sputum soiled articles

Funeral ceremonies in the houses of plague victims which may involve assembly of many people should be discountenanced as far as possible

CONTROL OF THE SPREAD OF PLAGUE AT DISTANCE

As will be gathered from the statements made in earlier parts of this monograph a spread of plague at distance may be effected

(1) by the arrival in a hitherto unaffected area of a plague patient who left an active focus when already ill or when incubating the disease

(2) by the transport of infected fleas on the persons or in the baggage of travellers,

(3) by the carriage of infected rodents and/or fleas in transport vehicles or in goods carried by these

As noted before the appearance of pneumonic plague in a hitherto unaffected locality was quite frequently due to the arrival of a bubonic plague patient who had developed lung complications en route Patients who fall ill with primary pneumonic plague while travelling and those who become ill after reaching their destination are equally apt to spread the infection in this form It is on the contrary an unlikely contingency that rodent infection will be produced in a hitherto plague free locality by the arrival of a bubonic patient who brought no infected fleas with him Importation of such fleas by human agency i e on the persons or in the baggage of travellers who may or may not have contracted plague themselves may take place and may lead to the infection of persons attacked by these fleas and/or to a transition of *P pestis* to the rodents On the whole however this mode of spread is not frequent particularly not as far as long distance spread is concerned

Carriage of infected rodents and/or fleas in transport vehicles or in export goods is of ominous importance for the spread of plague

In order to prevent the transport of bubonic plague infection by human agency it is sufficient as a rule (*a*) to ascertain the good state of health of the intending travellers through their medical inspection immediately before departure (*b*) to de flea their baggage with the aid of DDT calcium cyanide

or by other means, and (c) in the case of serious and/or not well controlled outbreaks, to apply DDT to these persons as is done for the purpose of controlling louse borne typhus The administration of plague vaccine to intending travellers, though not required by the international quarantine regulations,[236] ought to be given serious consideration as far as local traffic within or near plague affected areas is concerned However in order to be effective, vaccination of the intending travellers must be completed at least a week before their departure

While it is not difficult to adopt these procedures in regard to travellers intending to proceed by regular routes on larger ships or on trains or buses it is often most difficult to implement these methods in the case of local traffic by primitive means of communication However, the installation of temporary quarantine stations at strategic points round the affected localities is apt to remedy this situation to a considerable extent

In the case of pneumonic plague outbreaks it is as a rule quite sufficient to examine the intending travellers immediately before departure ascertaining at the same time that they have not been in contact with patients and have not lived in or near foci of the infection Persons who have had recent contact with pneumonic plague patients, and preferably also those suspected of such contact, ought to be kept under quarantine for 6 10 days before they are permitted to leave

It is quite easy to prevent the carriage of infected rodents and/or fleas in transport vehicles or in goods as long as one has to deal with the larger sea going ships As provided for in the international sanitary regulations,[36] sea going ships must be fumigated periodically unless because they are kept free from infestation through rat proofing procedures or regular anti rodent measures they have been granted exemption certificates Suspect goods to be carried by them may be fumigated before loading or may be disinfested after unloading on lighters or on shore

There ought to be no great difficulty in adopting similar procedures even on small vessels plying on inland waterways particularly those within or near plague foci—the less so because 1080 and calcium cyanide may be used in place of elaborate fumigation methods

DDT may be used to keep railway cars buses, and trucks free from fleas, while calcium cyanide is satisfactory for dealing with the goods carried by railways and trucks Again, however it is most difficult to control adequately the local traffic of goods apt to harbour infected fleas or even rodents particularly small grain consignments transported by primitive means of communication such as carts or pack animals, or carried by porters Temporary quarantine stations may prove useful in such cases but it is far more important to prevent goods destined for export from becoming infested by providing rat proof storage facilities for them As has been noted, in emergencies such goods may be kept on elevated platforms the supports of which are provided with rat guards

There can be no doubt however, that the most effective method of preventing the spread of plague at distance is to deal rapidly and drastically with the local manifestations of the infection Fully adequate methods are available for this purpose so that the task now confronting the plague workers is to apply these procedures intensively and universally

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228 Wanson M & Camphyn R (1949) *Ann Soc belge Med trop* 29 549
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ANNEXES

LIST OF RESERVOIRS AND VECTORS OF PLAGUE

Lists enumerating (*a*) the animals in respect of which positive proof of the infection has been obtained and (*b*) the suspected animals are given in tables I and II in order to show the occurrence of natural plague in rodent species or subspecies other than the commensal rats and mice and in Lagomorpha.

Particular attention has been paid to make table I not only as accurate as possible by closely following the standard nomenclature of Ellerman [30] and of Ellerman & Morrison Scott,[31] but as complete as possible. For its compilation valuable information has been derived from unpublished working documents generously made available by Macchiavello [99] and Davis [93].

These two documents as well as published material of Wu [201] and of Chabaud [0] were also used in the compilation of table V.

TABLE I. WILD RODENTS AND LAGOMORPHA PROVED NATURALLY INFECTED OR STRONGLY INCRIMINATED THROUGH POSITIVE FINDINGS IN THEIR ECTOPARASITES

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
BATHYERGIDAE	*Cryptomys* sp. White-toothed mole rat	Angola	38
CAVIIDAE			
Caviinae [a]	*Cavia aperea* Restless cavy	Brazil Ecuador	96 3
	Cavia pamparum Pampas cavy or Lund's guinea pig	Argentina	160
	Cavia tschudii atahualpae Peruvian cavy	Peru	99
	Caviella australis australis	Argentina	9
	Caviella australis joannia	Argentina	6
	Galea musteloides leucoblephara	Argentina	6
	Galea musteloides littoralis	Argentina	9
	Galea spixii Prea	Brazil	96
	Kerodon rupestris Brazilian rock cavy	Brazil	[illegible]

[a] The common guinea pig *Cavia porcellus* (*Cavia cobaya* auctt.), though repeatedly found plague-infected (Wu Lien-teh [ ] and others), has not been included in this annex since it is a domestic rather than a wild rodent. Referring to the findings recorded in Peru, Macchiavello stated that "plague infection in Cavia has been mentioned by several authors but without accurate taxonomic classification of specimens".

TABLE I (continued)

RODENTIA			
family and subfamily	species	Locality	Bibliographical reference
CHINCHILLIDAE	*Lagostomus maximus immollis* Vizcacha or Peruvian hare	Argentina	94
DIPODIDAE Dipodinae	*Allactaga elater* Small five toed jerboa	South east Russia	81
	Allactaga elater indica	Iranian Kurdistan	2
	Allactaga sibirica subsp (*A. saliens* auctt)	South east Russia	10
	Allactaga sibirica sibirica Mongolian five toed jerboa	Transbaikalia	170
	Dipus sagitta Northern three toed jerboa	South east Russia	187
ECHIMYIDAE Echimyinae	*Cercomys cunicularius laurentius*	Brazil	96
GEOMYIDAE	*Thomomys bottae* Western pocket gopher	California USA Colorado USA (?)	133 149
	Thomomys fossor (*T. talpoides fossor* auctt) Mountain pocket gopher	Fleas only Colorado	28
HETEROMYIDAE Dipodomyinae	*Dipodomys* sp	Texas USA	114
	Dipodomys ordi ordi Ord's kangaroo rat	Washington USA	109
Heteromyinae	*Perognathus* sp Pocket mouse	Fleas only Washington USA	138
	Heteromys anomalus anomalus	Venezuela	68
MURIDAE Cricetinae	*Akodon dolores*	Argentina	8
	Akodon mollis mollis	Peru Ecuador border region	99
	Akodon mollis orophilus Mountain field mouse	Huancabamba Peru	98 99
	Cricetus cricetus Common hamster	South east Russia	82
	Eligmodontia hirtipes jucunda	Argentina	8
	Eligmodontia moreni	Argentina	8
	Graomys griseoflavus centralis	Argentina	161
	Graomys griseoflavus griseoflavus	Argentina	9 1 93
	Hesperomys fecundus	Bolivia	09
	Hesperomys murillus cordovensis	Argentina	4

TABLE I (cont nued)

RODENTIA		Loca ty	B b o g aph cal efe ence
fam y and subfam y	spec es		
MURIDAE C ce nae (con nued)	*H l l l t alne r m*	Argent na	8
	Hol h l t s e s Suga cane at	B az l	96
	M on s alb a tal s Wh te ta ed at	South Af ca	190
	Neot ma alb gula a b gul	A zona USA New Me co USA	134 140 145
	Neot ma c ne n o lental s Wes e n bushy ta ed wood at	Ca fo n a USA	1 3
	Neo oma dese t um Desert wood at	Nevada USA Utah USA	109
	Neoton a f s pes Dusky footed wood at	Ca fo n a USA	04
	Neo ma f s pes n oha ens s Mohave Desert wood at	Nevada USA	109
	Ne toma nte med a nte med N lep da nte med a au tt) nte med ate Rhoads) wood at	Ca fo n a USA	13
	Neotoma lep da lep da	*Utah USA (?)*	111
	Neotoma m opus Pack at	Texas U A	114 [b]
	Ony homys sp	F eas on y Texas USA	14
	On homys leucogas er Wh te be ed g asshoppe mouse	F eas on y New Me x co and othe weste n a eas of the USA Te as	136 4
	Ony homys to dus	F eas on y New Mex co USA	13o
	O y omys an i nus	Pe u	99
	O y omys a enal s	Pe u	99
	O y o ys fla es ens subsp	A g nt na Bol a	8 99
	O y omys la eps nte me i u	B az	96 [c]
	O y omys la eps n dus	F eas on y Ecuado	99
	O y omys pal st s *Hesp e on ys p a u s a c t)*	New O eans La USA	107
	O y omys p haeopus ol us	Ecuado	99
	O mys s mann s mann *O l ng a i us s a n* au tt	Huancabamba Pe u	98 99

[b] Spontaneous plague n *Neot a* sp was reco ded n Oklahoma (Hampton) as well as in othe weste n parts of the USA

[c] Macch a e o a so ment oned nfect on in *O y r y m s* sp and *O y om s* sp (? s y a u)

TABLE 1 (continued)

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
MURIDAE Cricetinae (continued)	*Oryzomys xanthaeolus xanthaeolus*	Ecuador Peru *d*	99
	Oxymycterus sp (? *paramensis*)	Bolivia	99
	Peromyscus boylii	Fleas only Arizona USA	145
	Peromyscus leucopus	Fleas only New Mexico USA	145
	Peromyscus maniculatus	Fleas only California USA Washington USA	139 152
	Peromyscus truei gilberti Gilbert s white footed mouse	California USA	113
	Peromyscus truei truei True s white footed mouse	California USA New Mexico USA	111 *e* 18
	Phyllotis amicus maritimus	Fleas only Peru	99
	Phyllotis darwini vaccarum	Argentina	8
	Phyllotis fruticicolus	Ecuador	97
	Reithrodontomys megalotis Harvest mouse	Fleas only California USA Kansas USA New Mexico USA	139 137 147
	Rhipidomys sp (? *leucodactylus*)	Bolivia	99
	Rhipidomys equatoris	Peru	98 99
	Sigmodon hirsutus	Venezuela	68
	Sigmodon hispidus Cotton rat	Fleas and lice only New Mexico USA	49
	Sigmodon peruanus	Peru	99
Dendromyinae	*Dendromus haymani*	Belgian Congo	6
	Dendromus insignis kivu	Belgian Congo	26
	Malacothrix typicus Mouse gerbil	South Africa	191
	Steatomys pratensis	South Africa	183
Gerbillinae	*Desmodillus auricularis* Namaqua gerbil	South Africa	125
	Gerbillus paeba	South Africa	193
	Meriones libycus erythrourus	Iranian Kurdistan	2
	Meriones meridianus (*Pallasiomys merid* auctt) Midday gerbil	South east Russia Transcaspia Turkestan	186 44
	Meriones persicus persicus Persian jird	Iranian Kurdistan	2

d An unidentified subspecies of *Oryzomys xanthaeolus* was also found infected
e The presence of spontaneous plague in *Peromyscus* sp or their fleas was also repeatedly nfirmed (Eskey & Haas and others)

TABLE I (continued)

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
MURIDAE Gerbillinae (continued)	*Meriones [illegible] tristrami*	Iranian Kurdistan	2
	Meriones tamariscinus Tamarisk gerbil	South east Russia	44
	Rhombomys opimus Great gerbil	South east Russia	1[illegible]0
	Tatera brantsi (*T. l. lenguru* auctt.) Brants gerbil	South Africa	118 53
	Tatera indica Indian gerbil or antelope rat	India Iranian Kurdistan	40 2
	Tatera nigrita beniensis	Belgian Congo	6
	Tatera schinzi Schinz s gerbil	South Africa	35
Microtinae	*Ellobius lutescens*	Iranian Kurdistan	2
	Ellobius talpinus Northern mole vole	South east Russia	37
	Lagurus curtatus Sage brush vole	Fleas only Washington USA	144
	Lagurus lagurus Steppe lemming	South east Russia	80
	Microtus arvalis Common vole	South east Russia	22
	Microtus brandti Brandt s vole	Transbaikalia	170
	Microtus californicus Californian meadow mouse	California USA	134 f
	Microtus gregalis raddei Narrow skulled vole	Transbaikalia	170
	Microtus montanus	Fleas only Washington USA	146
	Microtus nanus	Fleas only Washington USA	14[illegible]
	Microtus socialis Social vole	South east Russia	81
	Microtus townsendi townsendi	Tacoma Wash USA	43
Murinae	*Acomys cahirinus* Cairo spiny mouse	Egypt	73 1[illegible]
	Aethomys kaiseri medicatus	Belgian Congo	6
	Arvicanthis abyssinicus sub[illegible]	Kenya	[illegible]4
	Arvicanthis abyssinicus nubila[illegible] Unstriped African grass rat	East Africa	13
	Arvicanthis abyssinicus [illegible]ssi	Belgian Congo	5 [illegible]

f Spontaneous plague in *Microtus* sp was also recorded on several occasions

TABLE 1 *(continued)*

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
MURIDAE			
Murinae (continued)	*Arvicanthis niloticus niloticus* Nile rat	Egypt	29
	Arvicanthis niloticus rufinus	Senegal	89
	Bandicota bengalensis bengalensis (*Gunomys bengal* auctt.) Lesser bandicoot rat	Burma India	52 58
	Bandicota bengalensis gracilis	Ceylon	56
	Bandicota bengalensis kok (*Gunomys kok* auctt.)	India	40
	Bandicota indica Large bandicoot rat	India	71 72
	Bandicota indica (*Bandicota malabarica* auctt.)	Ceylon	104
	Cricetomys gambianus Giant rat	Belgian Congo Gold Coast Senegal	26 45 87
	Dasymys incomtus bentleyae g Swamp rat	Belgian Congo	25 26
	Grammomys dolichurus	East Africa	95
	Grammomys dryas	Belgian Congo	5 26
	Lemniscomys striatus massaicus	East Africa	98
	Lemniscomys striatus striatus Spot striped grass mouse	Belgian Congo	26
	Lophuromys aquilus (*L. aquilus rita* auctt.)	Belgian Congo	25 26
	Millardia meltada Soft furred field rat or metad	India	162
	Mus booduga (*Leggada booduga* auctt.) Little Indian field mouse	India	40
	Mus deserti (*Leggada deserti* auctt.) Dwarf mouse	South Africa	183
	Mus musculoides emesi (*Leggada emesi* auctt.) Pigmy mouse	Belgian Congo	25 26
	Mus triton fors (*Leggada triton fors* auctt.)	Belgian Congo	25 26
	Mylomys cunninghami alberti (*M. dybowskii alberti* auctt.)	Belgian Congo	25 26
	Oenomys hypoxanthus hypoxanthus Rusty nosed rat	Belgian Congo	5 26

g Davis stated that *D. i. nudipes* [*Dasymys incomtus nudipes*] is found in the enzootic plague area in Barotseland, N. Rhodesia, where it is subject to secondary infection from gerbils and *Mastomys*

TABLE 1 (continued)

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
MURIDAE Murinae *(continued)*	*Pelomys campanae* h	Senegal	92
	Pelomys fallax iridescens	East Africa	95
	Rattus natalensis (*Mastomys coucha* auctt.) Multimammate rat	South Africa Kenya	53 118 54
	Rhabdomys pumilio Four-striped grass mouse	South Africa	117
Otomyinae	*Otomys* sp	East Africa	38
	Otomys angoniensis	Kenya	54
	Otomys irroratus South African water rat	South Africa	183
	Otomys tropicalis elgonis	Belgian Congo	5 26
	Otomys unisulcatus j (*Myotomys unisulcatus* auctt.) Karroo rat	South Africa	183
	Parotomys brantsi luteolus Eastern Karroo rat or Brants Otomys	South Africa	119
PEDETIDAE	*Pedetes cafer* South African spring hare	South Africa	119
SCIURIDAE	*Citellus armatus* Uinta ground squirrel	USA (western States) (Idaho Montana Nevada Washington Wyoming Utah)	21 108
	Citellus beecheyi beecheyi California ground squirrel	California USA	10? ?01
	Citellus beecheyi douglasi Douglas ground squirrel	California USA	108 131
	Citellus beecheyi fisheri Fisher s ground squirrel	California USA	108 109
	Citellus beecheyi nudipes	Fleas only California USA	60
	Citellus beldingi beldingi Belding s ground squirrel	California USA	133
	Citellus beldingi oregonus Oregon ground squirrel	California USA Oregon USA Nevada USA	107 33

[h] Garnham claimed that this rodent was afterwards identified as being *Lemniscomys griselda*

[i] Davis noted that in Barotseland *P. f. frater* [*Pelomys fallax frater*] is associated with *Otomys* and *Dasymys* and has a similar flea fauna It may act as a transient reservoir with these species

[j] In the list of plague affected wild rodents inserted in the 1936 *Manual* separate mention was made of three subspecies of *Otomys* (*Myotomys* auctt) To make the present list tally with that of Davis *Otomys unisulcatus* Cuvier and Geoffroy alone has been embodied

TABLE I (cont nued)

RODENTIA			
fam ly and subfam ly	spec es	Local ty	B bl o graph cal references
SCIURIDAE (cont nued)	*Citellus dauricus dauricus* Daur a s sel	Transba kal a	170
	Citellus columbianus columbianus Columb an ground squ rrel	Wash ngton USA Montana USA	111 148
	Citellus columbianus ruficaudis Blue Mounta n ground squ rrel	Oregon USA	111
	Citellus fulvus Large toothed susl k	South east Russ a	81
	Citellus idahoensis Idaho ground squ rrel	Fleas only Idaho USA	198
	Citellus lateralis chrysodeirus Golden mantled ground squ rrel	Cal forn a USA Colorado USA	109 ?8
	Citellus lateralis lateralis (*Callospermophilus lateralis* auctt) Say s ground squ rrel	Fleas only Wyom ng USA	137 k
	Citellus leucurus leucurus (*Ammospermophilus leucurus* auctt) Antelope ground squ rrel	Fleas only Ar zona USA Cal forn a USA	33 133
	Citellus mexicanus	Fleas only New Mex co USA	195
	Citellus pygmaeus L ttle susl k	South east Russ a	24 10
	Citellus richardsoni elegans Wyom ng ground squ rrel	Wyom ng USA Also Colorado Idaho Montana and Utah	18
	Citellus richardsoni nevadensis Nevada ground squ rrel	Nevada USA	111
	Citellus richardsoni richardsoni R chardson s ground squ rrel	Alberta Canada Montana USA Saskatchewan Canada	115 107 61
	Citellus spilosoma major	Fleas only New Mex co USA	141
	Citellus townsendi mollis P ute ground squ rrel	Fleas only Idaho USA Nevada and O egon USA	132 99
	Citellus tridecemlineatus 13 str ped ground squ rrel	Fleas only New Mex co USA Texas USA	135 114
	Citellus variegatus grammurus Say s rock squ rrel	Utah USA also fleas n A zo na Colorado and New Mex co USA	108
	Citellus variegatus utah Utah rock squ rrel	Utah USA	110

k Plague was also conf rmed in fleas from *Callospermophilus sp* In Cal forn a [1] and in fleas and ticks from *Citellus lateralis* In Colorado

TABLE I (continued)

RODENTIA family and subfamily	RODENTIA species	Locality	Bibliographical references
SCIURIDAE (continued)	*Citellus washingtoni loringi* Loring's ground squirrel	Washington USA	109 112
	Citellus washingtoni washingtoni Washington ground squirrel	Washington USA	109 112
	Cynomys sp	Fleas only Texas USA Colorado USA	 114 8
	Cynomys gunnisoni gunnisoni Gunnison prairie dog	New Mexico USA	141
	Cynomys gunnisoni zuniensis Zuni prairie dog	Arizona USA New Mexico USA	109
	Cynomys leucurus White tailed prairie dog	Fleas and lice only Wyoming USA	 109 33
	Cynomys ludovicianus Black tailed prairie dog	USA Colorado Kansas Montana New Mexico Texas Wyoming	134 150
	Cynomys parvidens Utah prairie dog	Utah USA	108
	Funambulus sp (? *F pennanti*)	South India	40
	Funambulus palmarum Indian palm squirrel	Ceylon India	55 167
	Glaucomys sabrinus lascivus Sierra Nevada flying squirrel	California USA	109
	Marmota bobak Bobak marmot or tarabagan	Manchuria Mongolia and Transbaikalia	11
	Marmota caudata Long tailed marmot	South east Russia	76
	Marmota flaviventris subsp Yellow bellied marmot	Colorado USA Fleas and lice Oregon USA Fleas New Mexico USA Fleas British Columbia Canada	130 133 93 6
	Marmota flaviventris avara	Fleas only Oregon USA	 130
	Marmota flaviventris engelhardti Engelhardt marmot	Montana Utah Wyoming USA	108 109
	Marmota flaviventris [illegible] Golden mantled marmot	Montana USA	109
	Marmota [illegible] baibacina (*Arctomys centralis* auctt)	Russian Turkestan	165 [illegible]
	Sciurus stramineus nebouxi Neboux squirrel	Ecuador Peru	99
	Tamias minimus (*Eutamias minimus* auctt)	Fleas only Washington USA	 [illegible]

TABLE I (concluded)

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
SCIURIDAE (continued)	*Tamias quadrivittatus frater* (*Eutamias speciosus frater* auctt) Tahoe chipmunk	California Nevada USA	108 109
	Tamiasciurus douglasi albolimatus Sierra Nevada chickaree	California USA	108 109
	Xerus erythropus Central African side striped squirrel	Senegal	87
	Xerus inauris inauris (*Geosciurus capensis* auctt) Bristly ground squirrel	South Africa	197
LAGOMORPHA			
LEPORIDAE	*Lepus californicus* Black tailed jack rabbit	California USA	133
	Lepus capensis [l] Cape hare	South Africa Argentina	183 8
	Lepus europaeus European hare	England	15 101
	Lepus saxatilis [l] Karroo hare	South Africa	175
	Lepus timidus Mountain or varying hare	Transcaspia	63
	Lepus zuluensis Zulu hare	South Africa	125
	Oryctolagus cuniculus [m] Rabbit	England	101
	Sylvilagus sp Cotton tail rabbit	Bolivia Ecuador Peru	99 99 98
	Sylvilagus andinus	Huancabamba Peru	98
	Sylvilagus audoboni	New Mexico USA	139
	Sylvilagus bachmani California brush rabbit	Fleas only (?) California USA	133
	Sylvilagus brasiliensis	Brazil	96
	Sylvilagus brasiliensis gibsoni	Argentina	178
	Sylvilagus nuttalli nuttalli Washington cotton tail rabbit	California USA	109

[l] Davis [33] stated in regard to these two hares that it is doubtful whether the records of first infections can be relied on to be zoologically exact *L. capensis* and *L. saxatilis* occur together over most of South Africa and extend northwards throughout the continent

[m] Instances of secondary plague manifestations among domesticated rabbits due to the presence of the infection among rats have been recorded by several workers e g recently by Buck et al who also referred to previous observations made in this respect in Madagascar

TABLE II WILD RODENTS AND LAGOMORPHA PRESUMED TO BE NATURALLY INFECTED

RODENTIA		Locality	Reference	Reason for non-inclusion in Table I[a]
family and subfamily	species			
CAVIIDAE Caviinae	*Galea* sp	Bolivia	99	B
DIPODIDAE Dipodinae	Jerboa (? *Jaculus* sp)	South east Russia	116	B
	Jerboa	Somaliland	100	B[b]
ECHIMYIDAE Echimyinae	*Ctenomys* sp (? *Ct. mendocinus*) Tucu tucu	Argentina	194 4	A B
MURIDAE Gerbillinae	*Gerbillus* sp	Tunis	42	A B
	Tatera valida liodon	Barotseland	23	A
Microtinae	*Arvicola* sp	Kenya	180	A B
	Brachytarsomys albicauda	Madagascar	90 158	A
	Microtus sp	Angola	106	A B
Murinae	*Aethomys chrysophilus*	South Africa	23	A
	Bandicota bengalensis varius (*Gunomys varius* auctt)	Calcutta India	85	B
	Saccostomus campestris Cape pouched rat	Nyasaland South Africa	86 23	A
	Thallomys nigricauda	South Africa	23	A
SCIURIDAE	*Citellus* sp *C. mongolicus umbratus* auctt)	South Manchuria	1	A B
	Marmota sp (? *M. himalayana robusta*	Tibet Kansu	168 1[illegible]3	A B
UNCLASSIFIED	Brush (coconut tree) rat	New Caledonia	129	A B
	Field mouse (mulot)	Azores	127 128	A B
		Khorassan	46	B
	Field rat	Rhodesia	79	B
		Tunis	4[illegible]	A B
LAGOMORPHA				
LEPORIDAE	*Lepus* sp	Bali	96	B
		French West Africa	8[illegible]	A B
		Russian Turkestan	76 77 1 1	A B

[a] A = incomplete evidence of infection
B = species undetermined or doubtful
[b] The presence of plague in other desert rodents was also suspected

TABLE III RODENTS AND LAGOMORPHA NOT FOUND NATURALLY INFECTED BUT PROVED SUSCEPTIBLE TO ARTIFICIAL INFECTION

RODENTIA		Locality	Bibliographical reference
family and subfamily	species		
BATHYERGIDAE	*Bathyergus suillus*	South Africa	23
	Cryptomys hottentotus	South Africa	23 125
DASYPROCTIDAE	*Dasyprocta aguti* Golden aguti (Cotia)	Brazil	166
	Dasyprocta prymnolopha Hairy rumped aguti	Brazil	166
DIPODIDAE Dipodinae	Jerboa (? *Jaculus* sp)	Egypt	189
	Jaculus orientalis Greater Egyptian jerboa	Tunis	197
ECHIMYIDAE Echimyinae	*Cercomys inermis* Punaré	Brazil	166
	Echimys lamarum Rata de espinho	Brazil	166
MURIDAE Cricetinae	*Cricetulus* sp (? *Cr barabensis griseus*) Striped hamster	China	59
	Cricetulus barabensis barabensis (*Cr furunculus* auctt)	Transbaikalia	69 176
	Cricetulus migratorius cinerascens (*Cr migratorius isabellinus* auctt)	Iranian Kurdistan	2
	Cricetulus (*Mesocricetus*) *eversmanni* Eversmann s hamster	South east Russia	44
	Mesocricetus auratus [a] Golden hamster	USA Iranian Kurdistan	105 2
	Sigmodon chonensis	Ecuador	99
Gerbillinae	*Gerbillus campestris* subsp	Tunis	197
	Gerbillus campestris dodsoni	Tunis	197
	Gerbillus pyramidum hirtipes	Tunis	197
	Meriones crassus charon	Iranian Kurdistan	2
	Meriones shawi grandis	Morocco	12
	Meriones shawi shawi	Tunis	197
	Psammomys obesus roudairei Fat sand rat	Tunis	197

[a] Baltazard et al [2] worked with *Mesocricetus auratus brandti* and *Mesocricetus auratus raddei* which they found as little susceptible to plague as the subspecies (? *Mesocricetus auratus auratus*) used by McMahon [1]

TABLE III (cont nued)

RODENTIA fam and sub am y	spec es	Loca y	B b o g aph ca ef nce
MURIDAE			
Ge b nae (cont nu d)	*Tate a af a*	Sou h Af ca	3
	Ta a e n	Kenya	1 7
M c o na	*1 l an pl l s* Wa e vo e	South east Russ a	41
	Apo l m ag ar St ped f e d mouse	Fo mosa	84
	A lem s at Common f e d mouse	Eu ope	1
	(ol n t ell l nd an bush a	nd a	40
	Len n om s l ba s 1 n h s ba b u a tt Ba bary s ped mouse o zeb a mouse	Tun s	197
	M om s m n s Harvest mouse	South east Russ a	44
	M ol s a Pe s an vo e	a an Ku d stan	2
	Th a l mys nan aqu s s [b]	Sou h Af ca	23
Tachyoryct nae	*Ta hy y tes l mon* O ange toothed mo e at	Cen a Af ca	95
MUSCARDINIDAE			
G aph u nae	*G aph u u* sp Do mouse (é o)	F ench Wes Af ca	9
	D yom ys n tedu Fo est do mouse	Sou h east Russ a	44
SCIURIDAE	*A n oze us get u*	Mo occo	12
	C tel us daur us mongol us Mongo an sus k	Manchu a	?07
	C l s dau us ramo us	Manchu a	1?
	C us undula us C e lus e e smann auctt Long ta ed S be an sus k	T an ba ka a	0
	e l s su l us gut utus Spo ted sus k	South east Rus a	163 164
	Ma m t n a A p ne ma mot	Eu ope	?08
	S u us s an eus s a eus	Ecuado	99

[b] *P p ls* was Iso ated from *X b a l ens s* found in a deserted nest of *Tha l mys n n a qu ns s*

TABLE III (concluded)

LAGOMORPHA		Locality	Bibliographical reference
family and subfamily	species		
LEPORIDAE	*Sylvilagus ecaudatus* Tapeti	Ecuador	99
OCHOTONIDAE	*Ochotona dauricus* Daurian pika	Transbaikalia	69 176

TABLE IV MAMMALS OTHER THAN RODENTS AND LAGOMORPHA KNOWN OR SUSPECTED TO BE NATURALLY INFECTED

Order	Species	Locality	Remarks	Bibliographical reference
ARTIODACTYLA	*Camelus* sp Camel	South east Russia	See chapter 6	
CARNIVORA	*Canis aureus* Asiatic jackal	India	Suspected by the Plague Research Commission	205
	Canis familiaris Domestic dog	French West Africa	Suspected	74
		Manchuria Morocco	See chapter 6	
	Cynictis penicillata Yellow mongoose	South Africa		25
	Felis catus Domestic cat	Most major plague areas	See chapter 6	
	Fox	Brazil	Suspected	19
	Herpestes sp Mongoose	India	Found infected	27
		Hawaii	One specimen found infected	47
	Mustela sp Weasel	Argentina		94
		USA western States		198
	Mustela altaica Alpine weasel	Iran an Kurdistan		2
	Mustela putorius European polecat	Near Odessa (south east Russia)		169
	Mustela putorius eversmanni	South east Russia		66

No mention has been made of the kangaroo because the solitary specimen found plague infected by Thompson had been involved in an outbreak among animals kept in the Sydney Zoological Gardens

TABLE IV (concluded)

Order	Species	Locality	Remarks	Bibliographical reference
CARNIVORA (continued)	*Mustela putorius furo* Ferret	South Africa	Plague was confirmed in ferrets used to catch rats at Port Elizabeth	05
	Mustela sibirica itatsi Siberian weasel	Japan	One specimen found infected	63a
	Mustela vison Mink	Canada	Incriminated	41
	Suricata suricatta Suricat	South Africa	Suspected	1 5
	Taxidea taxus neglecta Western badger	Oregon USA	Found infested with plague infected ticks	133
INSECTIVORA	*Crocidura olivieri* (*Cr stampflii* auctt) Egyptian giant shrew	Senegal		91
	Erinaceus sp Hedgehog	Senegal	Found infected	74
	Suncus murinus (*Crocidura caerulea* auctt) House shrew [a]	India		05
		Formosa		205
		Cambodia		78
		China		Pollitzer (unpublished reports)
	Sylvisorex gemmeus irene	Belgian Congo		26
MARSUPIALIA	*Monodelphis domesticus* (*Peramys domesticus*) Opossum	Brazil		99
	Didelphis aurita	Brazil	Found infested with infected fleas	99
PERISSODACTYLA	*Equus* sp Donkey [b]	Manchuria		[illegible]
PRIMATES	*Presbytis entellus* Langur	India		[illegible]
	Macaca radiata Bonnet monkey [c]	India		[illegible] 51

[a] According to Bangxang shrews also played a role in the plague outbreaks of Thailand
[b] Strong & Teague who found donkeys insusceptible to plague infection by inhalation doubted the validity of Fujinami's findings
[c] According to Sáenz Vera monkeys were possibly also involved in Ecuador

TABLE V WILD RODENT FLEAS FOUND NATURALLY INFECTED OR PROVED TO BE SUSCEPTIBLE TO EXPERIMENTAL INFECTION

Species	Locality	Usual hosts	Findings *	Bibliographical reference
Amphipsylla rossica	South east Russia	*Lagurus* *Microtus*	E	67
Anomiopsyllus sp	New Mexico USA	*Neotoma*	S	153
Anomiopsyllus hiemalis	Texas USA	*Neotoma*	S	114
Anomiopsyllus nudatus	USA western States	*Neotoma*	E	33
Atyphloceras sp	USA western States	*Lagurus* *Peromyscus*	S^p	153
Atyphloceras multidentatus	USA western States	*Neotoma* *Microtus* *Peromyscus*	E T	33
Catallagia decipiens	Washington USA	*Lagurus* *Peromyscus*	S^p	153
Catallagia wymani	USA western States	*Microtus* *Neotoma* *Reithrodontomys*	E	33
Cediopsylla spillmanni	Huancabamba Peru	*Sylvilagus*	S	99
Chiastopsylla rossi	South Africa	*Otomys* *Rhabdomys* *Rattus*	E T X	65
Citellophilus tesquorum	South east Russia Transbaikalia	*Citellus*	E T X	44
Craneopsylla wolffhugeli	Argentina	*Graomys* and other rodent	S	8
Ctenophthalmus breviatus	South east Russia	*Citellus* *Microtus*	E	44
Ctenophthalmus cabirus	East Africa Belgian Congo	*Arvicanthis* and other rodents	E T S T	179 96
Ctenophthalmus orientalis	South east Russia	*Citellus* and other rodents	E	44
Ctenophthalmus phyris	Belgian Congo	*Arvicanthis* *Lemniscomys* *Otomys*	S	26
Ctenophthalmus pollex	South east Russia	*Citellus* *Arvicola*	S	189a
Ctenophthalmus wagneri	South east Russia	*Cricetus*	E	184
Delostichus (Parapsyllus) talis	Argentina	*Cavia*	S T X	47 178
Diamanus montanus	USA western States	*Citellus*	S T X	33 34 199 200

* E = experimentally infected
S = found naturally infected
S^p = found together with wild rodent fleas belonging to other species in pools which proved plague positive
T = transmitted plague in the laboratory
X = known to bite man

TABLE V (continued)

Species	Locality	Usual hosts	Findings	Bibliographical reference
Din r yllus ell bius ell biu (D l p usus auctt)	South Africa	*Rhabdomys Tatera* and other rodents	S X	65
Din p yllu ell bius lypu us	East Africa Belgian Congo	*Arvicanthus* and other wild rodents	S T X	25 34 1 6 157
Foxella ignota	Colorado USA	*Thomomys*	S	8
Front p ylla emura	South east Russia	*Citellus*	E X	44
Hect p ylla e legi	Peru	*Cavia* and rats	S T X	99
H l p ylla suire i	Ecuador	*Cavia Rattus*	S X	175
Hopl p yllus anden is	Huancabamba Peru	*Sylvilagu*	S	99
Hoplop ylla anom ilus	USA western States	*Citell is*	S T X	34 103
Hoplop yllus erotucus	Huancabamba Peru	*Sylvilagus*	S	99
Hoplopsyllus glacialis affinis (H affinis auctt)	New Mexico USA	*Sylvilagu*	S	153
Hy trichopsylla dippi i	USA western States	*Citellu* and other wild rodent	E T	33 198
Listrop ylla sp	South Africa		S	172
Malaraeus telchinum	USA western States	*Microtu Per m y u*	E T	16 33
Megabothris abantis	USA western States	*Mi rot s* and other wild rodents	E T	17
Megabothris clant ni	Washington USA	*Lagurus Perom ys us*	S[P]	1 3
Megarthroglossus divisus (M l ngi spinus auctt)	USA western States	*Neotoma Tamias*	E	33
M ringis sl ann ni	Washington USA	*Lagurus* and other wild rodents	S[P]	1 3
Mon p yllus cili tus	USA western States	*Tamias Ta mia ciurus*	E	33
M nop yllus eum lpi	USA western States	*Ta ui*	S T	33 1 3
Mor opsyllus exilis	Texas USA	*O ych my*	S	114
M n p yllus wagneri	USA western States	*L giru Per y cus* and other wild rodent	S[P]	154
Ne psylla i iopina	USA western States	*Citellu*	E	33
Neop ylla set a	South east Russia	*Citellu* and other wild r d nt	S T X	44
N pyll eras ro enbergi	Ecuador	*Didelphi* and wild r d t	S	99

TABLE V (continued)

Species	Locality	Usual hosts	Findings *	Bibliographical reference
Nosopsyllus sp	Iranian Kurdistan	*Meriones*	S X	2
Nosopsyllus consimilis	South east Russia	Mice	E T X	44 184
Nosopsyllus laeviceps	South east Russia	*Lagurus*	E X	44
Nosopsyllus mokrzeckyi	South east Russia	Mice	S T X	44 188
Nosopsyllus nilgiriensis	South India	*Bandicota*	E (suspected as vector)	39 64
Odontopsyllus sp	Huancabamba Peru	*Sylvilagus*	S	99
Opisocrostis bruneri	USA western States	*Citellus*	E T	126
Opisocrostis hirsutus	USA western States	*Cynomys*	S T	33 153
Opisocrostis labis	USA western States	*Citellus*	E T	33
Opisocrostis tuberculatus	USA western States	*Citellus* *Cynomys*	E T	33
Opisodasys nesiotus	California USA	*Peromyscus*	E T	17
Orchopeas sexdentatus sexdentatus	USA western States	*Neotoma*	S T	33 153
Oropsylla idahoensis	USA western States	*Citellus*	E	33
Oropsylla rupestris	USA western States	*Citellus*	E T	33
Oropsylla silantiewi	Manchuria Mongolia Transbaikalia	*Marmota*	S T X	177 206
Pleochaetis sp	Huancabamba Peru	*Akodon* *Oryzomys*	S T	99
Plocopsylla hector	Ecuador	*Thomasomys* and other wild rodents	S	99
Polygenis sp	Ecuador Raqui Peru Huancabamba Peru	*Akodon* *Akodon* *Oryzomys*	S S S	99 99 203
	Venezuela	*Heteromys* *Sigmodon* rats	? S	68
Polygenis gwyni	USA southern and southwestern States	*Sigmodon* *R. norvegicus*	E T	57
Polygenis litargus	Ecuador Peru border region Huancabamba Peru	*Sciurus* *Oryzomys* *Akodon*	S T	98 99
Polygenis platensis cisandinus	Argentina	*Oryzomys*	Suspected as vector	98
		Cavia and other wild rodents	Suspected as vector	98
Thrassis acamantis acamantis	USA western States	*Marmota*	E T	33
	Canada	*Marmota*	? S	62

TABLE V (concluded)

Species	Locality	Usual hosts	Findings	Bibliographical reference
Thrassis acamantis howelli	USA western States	*Marmota*	E T	33
Thrassis arizonensis	USA western States	*Citellus*	E T	33
Thrassis bacchi bacchi (= *Thr. gladiolis*)	USA western States	*Citellus*	S T	126 153
Thrassis bacchi johnsoni	USA western States	*Lagurus* *Peromyscus*	SP	1 3
Thrassis fotus	USA western States	*Onychomys*	S T	1 3 199
Thrassis francisi	USA western States	*Citellus*	E T	33
Thrassis pandorae	USA western States	*Citellus*	E T	33
Thrassis petiolatus	USA western States	*Citellus*	E	33
Tiamastus cavicola (*Rhopalopsyllus cavicola* auctt.)	Ecuador Peru	*Cavia*	S T	99
Tritopsylla intermedia cephala	Ecuador		S	99
Xenopsylla sp. (*conformis* group)	Iranian Kurdistan	*Meriones*	[illegible] X	
Xenopsylla sp. (*X. mycerini* auctt.)	South east Russia	*Meriones*	E X	44
Xenopsylla eridos (*X. pastylae*)	South Africa	*Otomys* and other wild rodents	Probably spontaneously infected	23
Xenopsylla hirsuta	South Africa	*Tatera* and other wild rodents	E T	171
Xenopsylla philoxera (*X. eridos* auctt.)	South Africa	*Tatera* and other wild rodents	S T	65
Xenopsylla phyllomae	South Africa	*Desmodillus* and other gerbils	S X	172
Xenopsylla piriei	South Africa	*Desmodillus*	S	[illegible]
Xiphiopsylla lippa	Belgian Congo	*Lophuromys* and other wild rodents	S	[illegible]

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Annex 2

IDENTIFICATION OF FLEAS

F G A M SMIT

Custodian of the Rothschild Collection of Siphonaptera
British Museum (Natural History)
Zoological Museum Tring Hertfordshire England

Fleas are small (1-8 mm long) wingless insects with a holometabolous metamorphosis (egg—larva—pupa—adult) In the adult stage only, and generally temporarily, they parasitize (i e, suck blood from) mammals and birds Their bodies are strongly laterally compressed, usually heavily sclerotized hairy and shiny, light rufous to almost black The larvae are apodous elongate, strongly haired, and eyeless, but have biting mouth parts, they are not parasitic, but feed on organic matter which they mainly find in their usual abode the nest of the host The free pupa is enveloped by a cocoon

At present the number of described species and subspecies of fleas is approximately 1,400, but only a minority of these figure largely in the literature on plague

According to the latest classification the order of fleas, Siphonaptera, is divided into two superfamilies Pulicoidea and Ceratophylloidea The former superfamily though the smaller, contains most of the better known fleas e g, the genera *Pulex*, *Xenopsylla* *Echidnophaga*, and *Ctenocephalides* while the latter superfamily includes *Leptopsylla* *Nosopsyllus* and *Monopsyllus* The two superfamilies together are divided into 17 families [a]

The object of this note is (*a*) to show the way of preparing fleas for study and identification, and (*b*) to point out pictorially the more important and conspicuous taxonomic characters of fleas so as to make intelligible the illustrated key (see page 650) for the prima facie diagnosis of those fleas which are predominant in medical literature [b]

Preparation of Fleas for Study and Identification

The specimens immediately after their capture are preserved in small tubes with 70% 80% alcohol or methylated spirit in which they may be stored indefinitely If storage is to be for a long period a drop of glycerin

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[a] For details of this classification, and a key to all families see Vol I of the *Catalogue of the Rothschild collection of fleas* by G H E Hopkins & M Rothschild published by the British Museum (Natural History) London

[b] When there is any doubt as to the correctness of a determination specimens should be sent for verification to a specialist they would be welcomed by The Keeper Department of Entomology British Museum (Natural History) Cromwell Road London S W 7

may be added. Never use a formaldehyde solution as a preservative fluid. Adequate labelling of the tube is most important.

Fleas can only be studied and identified satisfactorily if they are mounted properly. The following method is still the best known. Pass the specimens (which should be kept in the same tube while changing the fluids; pin the label on the cork) successively through the following liquids:

(*a*) Water: one hour.

(*b*) 20% solution of potassium hydroxide at room temperature: one or more (generally two) days, till the specimens are yellowish or light rufous and somewhat transparent.

(*c*) Water: a few minutes.

(*d*) 5% aqueous solution of glacial acetic acid: half an hour.

(*e*) Water (renew once): one hour.

If permanent mounts are not desired, the specimens can at this stage be studied on a slide in water (under a coverslip) and afterwards returned to a tube containing alcohol. For making permanent mounts, the next steps are the following:

(*f*) Put the specimens on a clean slide a short distance from each other (taking care to keep the batches apart). Remove with blotting paper any superfluous water round the specimens but avoid drying up. Arrange—with the aid of two, very fine mounted needles under a dissecting microscope (or a powerful lens)—the legs in proper position, i.e., downwards and free from each other, as in fig. 1 (see page 655). Put on a coverslip and let absolute alcohol run from a small pipette underneath the slip (95% alcohol or dehydrated methylated spirit may be used). Since the alcohol evaporates quickly from underneath the coverslip, some more alcohol should be added regularly. Very large and thick fleas should be covered with half a slide, since the pressure of a coverslip would not be sufficient; leave for half an hour under coverslip.

(*g*) Take the specimens off the slide and put them in absolute alcohol: one hour or longer.

(*h*)[c] Oil of cloves (oleum caryophyllorum): at least for some hours, preferably for a day or longer; at first the specimens will float on the oil but eventually they will sink (if not, they should be pushed into the oil with a fine needle).

(*i*) Xylol (renew once): about 10 minutes.

(*j*) Mount the specimens in Canada balsam dissolved in xylol. Every flea should be put on its right hand side on the slide and it is advisable not to place more than one specimen under one coverslip.

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[c] If one prefers to use euparal as a mounting medium (because of its colourlessness and more favourable refractive index) the procedure is the same up to (*g*) inclusive; one then proceeds as follows:

(*h*) Eucalyptol: one day.

(*i*) A mixture of 1 part of eucalyptol and 1 part of euparal: one day.

(*j*) Mount the specimens in euparal and use this medium also for ringing, if so desired.

(*k*) Dry the slides on a hot plate or in an oven at 80° 90°C for about half an hour

(*l*) After some days, the slides may be ringed, if circular coverslips have been used, with Canada balsam, dry again at 80° 90°C, but only for about 10 minutes

(*m*) Label the slides well The data to be mentioned are name of the host (preferably its scientific name) locality, altitude, date, and name of the collector, after identification, the name of the flea should be added

One should always handle fleas with care and, for instance, lift them out of fluids with a lanceolate mounted needle or a similar instrument, so as not to damage specimens through loss of setae

Illustration of Taxonomic Characters of Fleas

The more important taxonomic characters of fleas have been illustrated in fig 1 40 in order to facilitate interpretation of the key to the identification of some common fleas (see below) Nearly all the characters mentioned in this key are shown in the figures, the drawings were not all made to the same scale A list of abbreviations of technical terms used in the key and in figures 1, 2 5, 6 11, 15 25, and 26 is given below

Abbreviation	*Explanation*
a	abdomen
a ap	aedeagal apodeme (also called penisplate)
a s	antesensilial seta
a st	anal stylet of female
ac s	acetabular seta
an	antenna
ap t IX	apodeme of tergum IX of male
b c	bursa copulatrix
c	coxa
cl	claw of tarsus
co	corpus of clasper
ct	ctenidium (a comb of spines)
d a st IX	distal arm of sternum IX of male
d b c	ductus bursae copulatricis
d o	ductus obturatorius (blind duct)
d s	ductus seminalis (duct of spermatheca)
e	eye
ep	epipharynx (an unpaired structure)
F	movable process of clasper
f	femur
fr	frons
g ct	genal ctenidium
h	head
l	lacinia
l p	labial palp
m	manubrium
m p	maxillary palp
mse	mesepimeron
mses	mesepisternum
msn	mesonotum
mss	mesosternum
mte	metepimeron
mtes	metepisternum
mtn	metanotum
mts	metasternum
o	occiput
o s	ocular seta
P	process of the corpus of clasper
p	pronotum
p a st IX	proximal arm of sternum IX of male
p ct	pronotal ctenidium
pl r	pleural rod of mesopleurum
r sp	reservoir of spermatheca
s	sensilium
s f	spiracular fossa
sp	spermatheca (also called receptaculum seminis)
st	sternum (sterna II X)

Abbreviation	Explanation	Abbreviation	Explanation
sti	stipes (also called maxilla or maxillary lobe)	ta	tarsus
		th	thorax
t	tergum (terga I–X)	ti	tibia
t sp	tail of spermatheca (also called appendix)	tr	trochanter

Key to Identification of Some Common Fleas

1 Without ctenidia — 2
At least pronotum with a ctenidium — 6

2 Mesosternosome without an internal pleural rod (fig 8) — 3
Mesosternosome with an internal pleural rod (fig 1 and 15, pl r) — 4

3 Frons angulate (fig 3) genal lobe directed backwards laciniae very broad and coarsely serrate occiput with two setae and usually with a well developed lobe in the female only thorax dorsally narrower than tergum I (fig 3) fifth tarsal segment with three pairs of stout equally spaced lateral setae and a smaller fourth pair, and with two subapical plantar setae (fig 4) claws of tarsus without a large basal projection (fig 4) ♂ processes F 1 and F 2 not quite reaching to the middle of process P, the latter having a long and downward pointing apical seta (fig 5) ♀ sternum VII and spermatheca (rather variable in shape) as shown in fig 6 *All zoogeographical regions most common* in the Ethiopian region absent from the cooler areas including for example nearly the whole of Europe and also absent from the greater part of the neotropical region on poultry and other birds and on a large variety of small mammals A stick tight flea i e the female buries its piercing mouthparts in the skin of the host and is not easily removed

Echidnophaga gallinacea (Westwood) 1875

Frons smoothly rounded (fig 7) ocular seta placed below the conspicuous eye (fig 7 cf fig 13 14) one small spinelet at the genal margin (seldom two sometimes absent) occiput with only one strong seta ♂ with a broad process P covering the processes F 1 and F 2 (fig 9) spermatheca with a globular reservoir and a curved tail (fig 10) outline of sternum VII as in fig 10 but usually hardly visible in mounted specimens Cosmopolitan on man and other mammals particularly pig and badger

Pulex irritans Linne 1758 [d]

4 ♂ antesensilial seta on a marginal cone (fig 11) process P with seven or eight strong setae one of which is stouter than the others and

[d] The genus *Pulex* contains only two other species *P. porcinus* Jordan & Rothschild known from the southern part of the Nearctic region and *P. oculus* Traub from Guatemala

precedes an elbowed seta (fig 11), process F longer than process P and slightly bent upwards towards its apex (fig 11), sternum IX straight, with only a few small setae along its ventral margin (fig 11), aedeagal apodeme rather narrow its ventral margin not undulate (fig 20)

♀ antesensilial seta not marginal (fig 12), sternum VII as in fig 12 reservoir of spermatheca for the greater part almost globular and much wider than the base of its tail (fig 12) Originally an Ethiopian species, but has now been found in many other parts of the world, mainly on rats

Xenopsylla brasiliensis (Baker) 1904

Antesensilial seta of both sexes not marginal (fig 16 and 17), reservoir of spermatheca either not globular or much smaller than the base of its tail (fig 17 and 19) 5

process P rather broad with a straight or slightly concave apical margin and a number of fairly slender setae (fig 16) process F with its tip a little curved downwards never upwards (fig 16), sternum IX straight and widened towards the apex which is not much or hardly at all turned upwards (fig 16), aedeagal apodeme with a concave dorsal margin and broadest pre apically, its ventral margin partly sinuate (fig 21)

♀ sternum VII as in fig 17 reservoir of spermatheca somewhat longer than broad, not broader than the base of its tail, while the lower margin of head and tail are about level (fig 17) Cosmopolitan principally on rats

Xenopsylla cheopis (Rothschild) 1903

♂ process P not much widened apically with six to nine slender setae along its dorso apical margin (fig 18), process F longer than process P (fig 18) sternum IX with a strongly sclerotized ventro marginal strip bearing at its apical half a number of minute setae (fig 18) aedeagal apodeme with a rounded apex much wider pre apically than basally (fig 22)

♀ sternum VII as in fig 19, reservoir of spermatheca smaller than the basal part of its tail which is strongly ventricose its outline being almost spherical (fig 19) Oriental region, introduced on East African coast on gerbils, also on rats

Xenopsylla astia Rothschild 1911

6 With a ctenidium on pronotum only (fig 23) 7

With a ctenidium on head and one on pronotum (fig 1, 32 34) 8

7 No setae in front of the row of three below the eye but one or a few small ones above this row (fig 23 27) first hind tarsal segment besides the lateral setae with a row of setae on the outer surface none of the

apical setae of the second hind tarsal segment reaches the apical margin of the third segment

♂ sternum VIII vestigial (fig 25) tergum VIII strongly rounded from behind the last dorsal marginal seta (fig 24) manubrium shorter than (or at most as long as) the clasper the latter measured from the apex of the angle formed by the apodeme of tergum IX and the manubrium (this angle is very obtuse) to the pair of acetabular setae (fig 25) clasper and sternum IX as in fig 25

♀ bursa copulatrix long and spirally shaped (fig 26 b c) tail of spermatheca not narrowed towards its apex (fig 26) posterior margin of sternum VII slanting not incurved (fig 26) Cosmopolitan on a large variety of rodents, chiefly on rats

Nosopsyllus fasciatus (Bosc) 1800

Head very much like that of *N fasciatus* (cf fig 23 and 27) no setae of hind tarsal segments I and II extending beyond the segment following

♂ sternum VIII long and narrow usually with a very narrow membranous pre apical process (fig 28) anterior margin of tergum IX forming with the manubrium an angle much smaller than 90° this angle being rounded off (fig 28) process P conical bearing one long and two short setae (fig 28) process F elongate proximal arm of sternum IX strongly bent (fig 28)

♀ posterior margin of sternum VII rounded (fig 29) head of spermatheca cylindrical about three times as long as the tail (fig 29) Temperate eastern Asia on rats

Monopsyllus anisus (Rothschild) 1907

8 Genal ctenidium vertical, consisting of four spines (fig 32) two of the setae near the frontal angle are spiniform (fig 32) clasper as in fig 30 sternum VII and spermatheca as in fig 31 The tibiae bear at their dorso posterior margin a row of spiniform setae which form a so called false comb Cosmopolitan mainly on the house mouse though in certain areas common on rats

Leptopsylla segnis (Schönherr), 1811

Genal ctenidium horizontal generally consisting of eight to nine spines (fig 33 and 34) 9

9 Head strongly rounded anteriorly in both sexes first spine of genal ctenidium about half as long as the second (fig 33) hind tibia with the setae A and B as shown in fig 35 ♂ manubrium with a dilated apex (fig 37) ♀ apical part of the tail of spermatheca long (fig 39 cf fig 40) Cosmopolitan though rare in the Oriental and Ethiopian regions mainly on Canidae

Ctenocephalides canis (Curtis) 1826

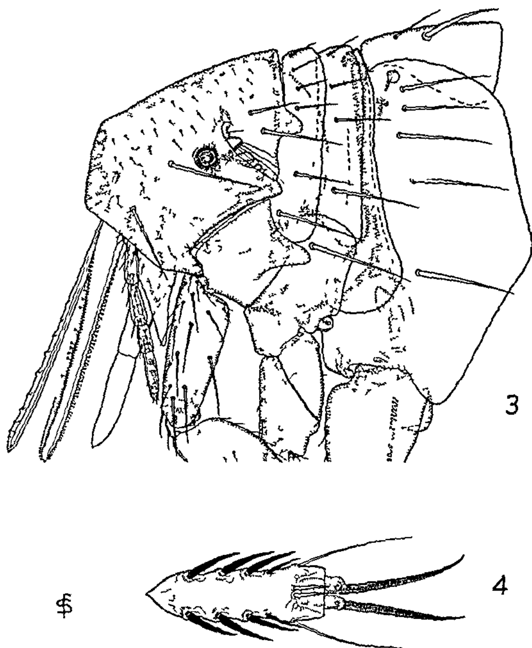
Head not strongly convex anteriorly [e] hence more elongate than that of *Ct canis* (fig 1, 2 and 34) first spine of genal ctenidium about as long as the second, hind tibia only with seta A, seta B absent, though rarely represented by a minute seta (fig 36), ♂ manubrium only a little dilated (fig 38), ♀ apical part of the tail of spermatheca short (fig 40, cf fig 39) Cosmopolitan, on a large variety of (preferably fairly large) mammals

Ctenocephalides felis felis (Bouche), 1835

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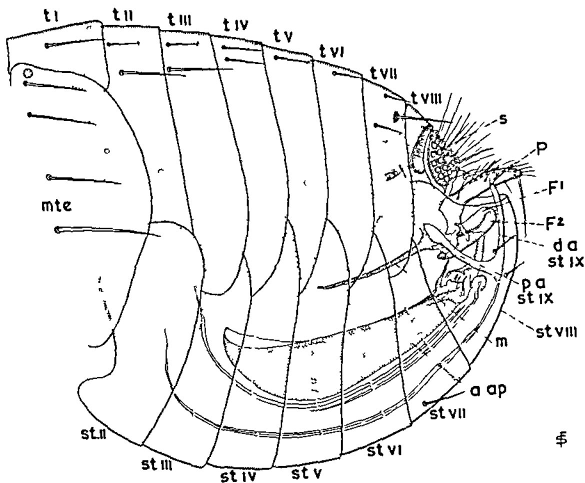
[e] In the subspecies *felis strongylus* (from Africa) and *felis orientis* (occurring from Ceylon to the Admiralty Islands but not in Australia) the head is short and not unlike that of *Ct canis* in *felis damarensis* (south west Africa) it is more like that of the nominotypical subspecies

FIG 3 4 ECHIDNOPHAGA GALLINACEA (WESTWOOD)



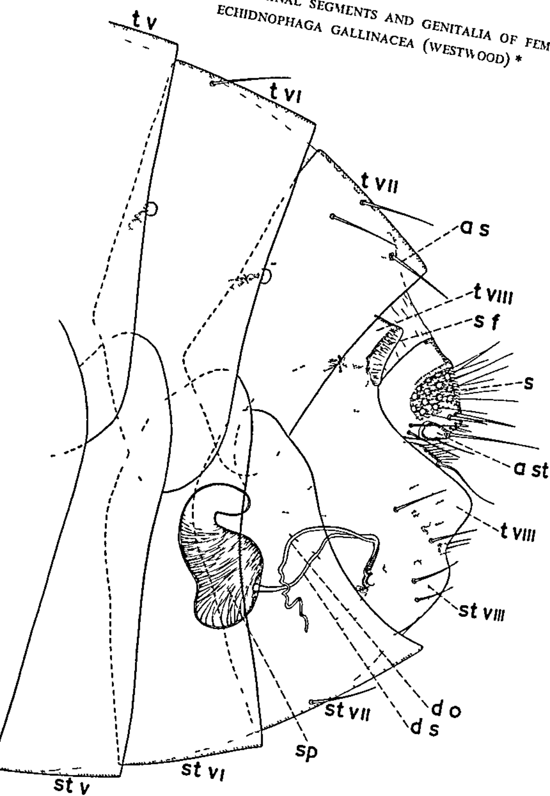
3 Head thorax and tergum I ♀ *4 Last hind tarsal segment,* ♀

FIG 5 ABDOMEN OF MALE ECHIDNOPHAGA GALLINACEA (WESTWOOD) *



* For explanation of lettering see page 650

FIG 6 TERMINAL SEGMENTS AND GENITALIA OF FEMALE ECHIDNOPHAGA GALLINACEA (WESTWOOD) *



* For explanation of lettering see page 650

FIG 7 HEAD, PROTHORAX AND FORE COXA OF MALE PULEX IRRITANS LINNE

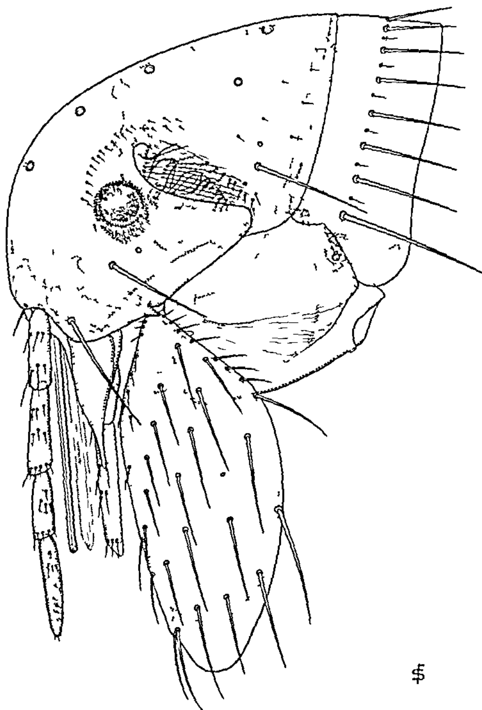
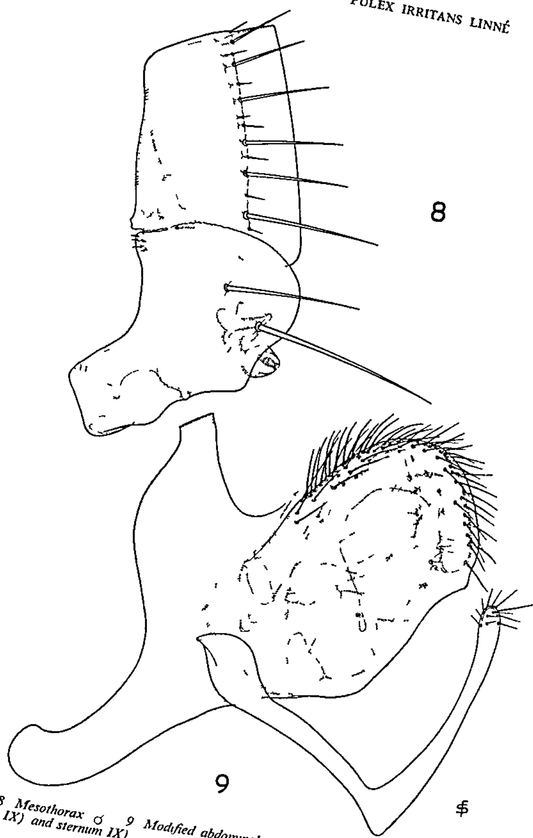


FIG 8 9 PULEX IRRITANS LINNÉ



8 Mesothorax ♂ 9 Modified abdominal segment IX ♂ (i e clasper (= tergum IX) and sternum IX)

FIG 10 TERMINAL SEGMENTS AND GENITALIA OF FEMALE PULEX IRRITANS LINNE

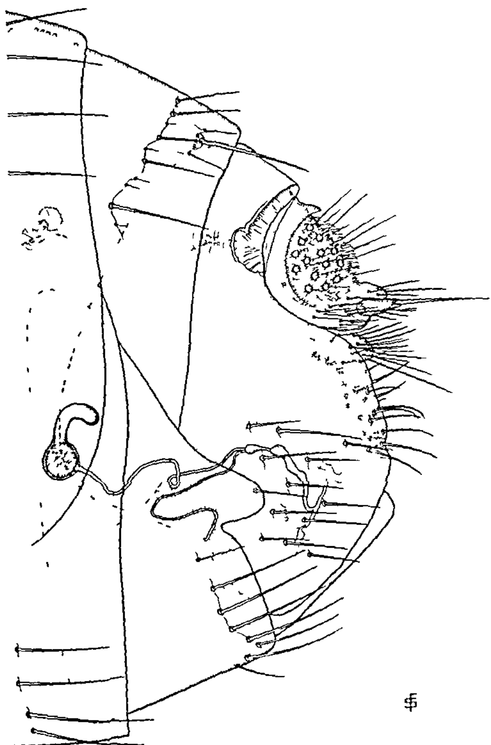
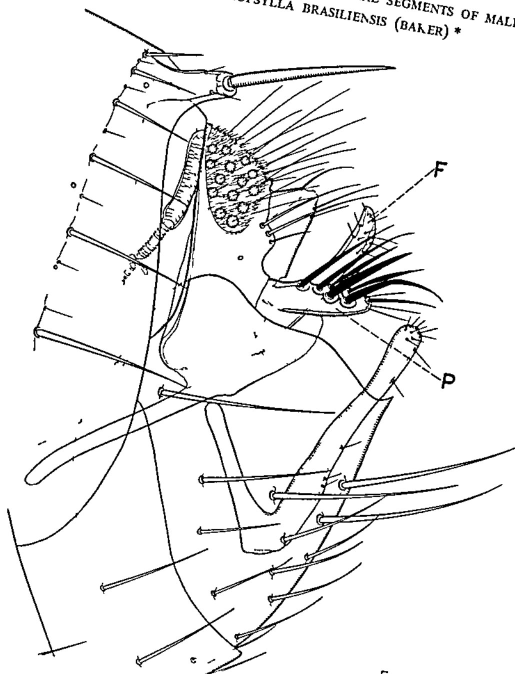


FIG 11 MODIFIED ABDOMINAL SEGMENTS OF MALE XENOPSYLLA BRASILIENSIS (BAKER) *



* For explanation of lettering see page 650

FIG 12 TERMINAL SEGMENTS AND GENITALIA OF FEMALE XENOPSYLLA BRASILIENSIS (BAKER)

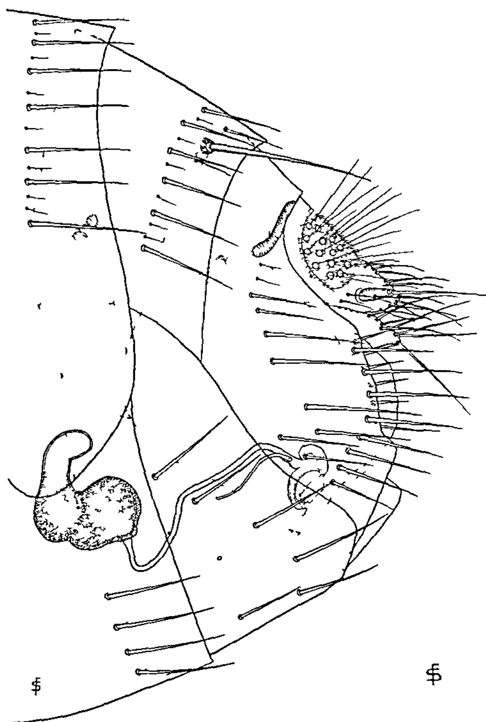
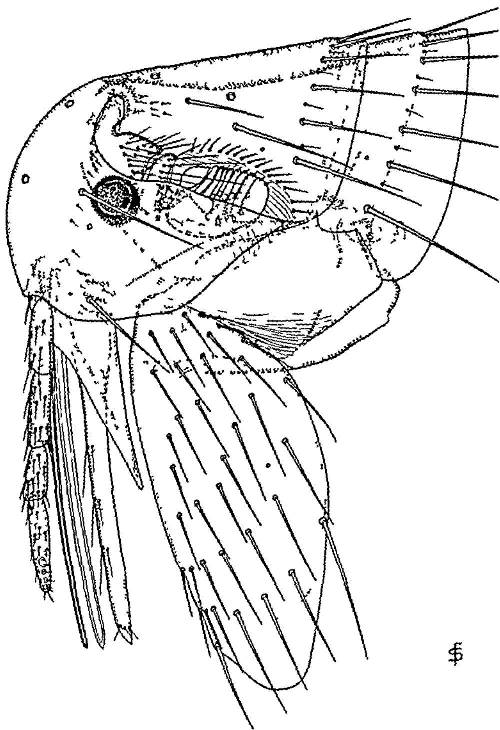
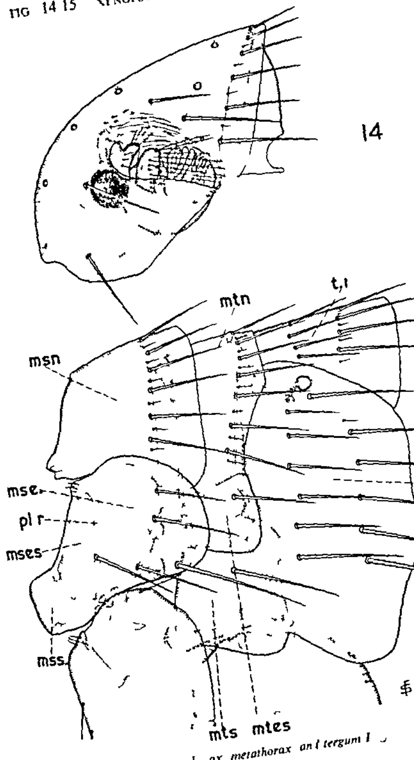


FIG 13 HEAD, PROTHORAX, AND FORE COXA OF MALE XENOPSYLLA CHEOPIS (ROTHSCHILD)



ANNEX 2

FIG 14 15 XENOPSYLLA CHEOPIS (ROTHSCHILD) *



I ax metathorax and tergum I

FIG 16 MODIFIED ABDOMINAL SEGMENTS OF MALE XENOPSYLLA CHEOPIS (ROTHSCHILD)

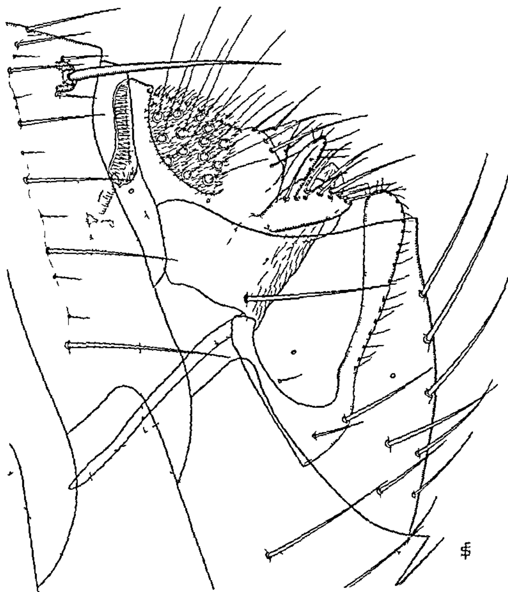


FIG 17 TERMINAL SEGMENTS AND GENITALIA OF FEMALE XENOPSYLLA CHEOPIS (ROTHSCHILD)

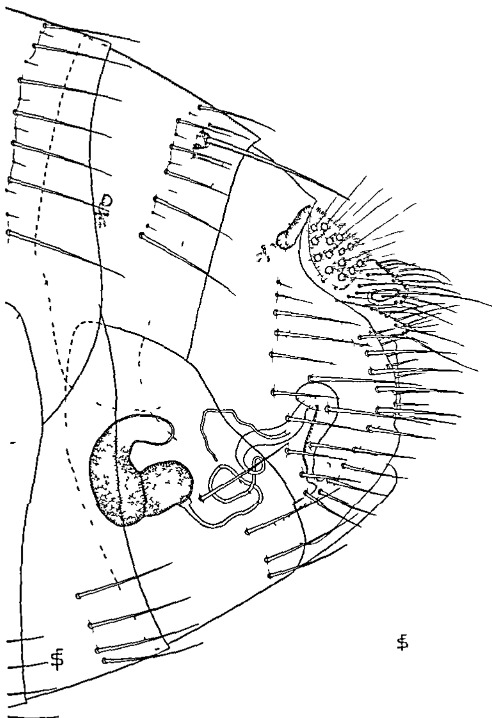


FIG 18 MODIFIED ABDOMINAL SEGMENTS OF MALE XENOPSYLLA ASTIA ROTHSCHILD

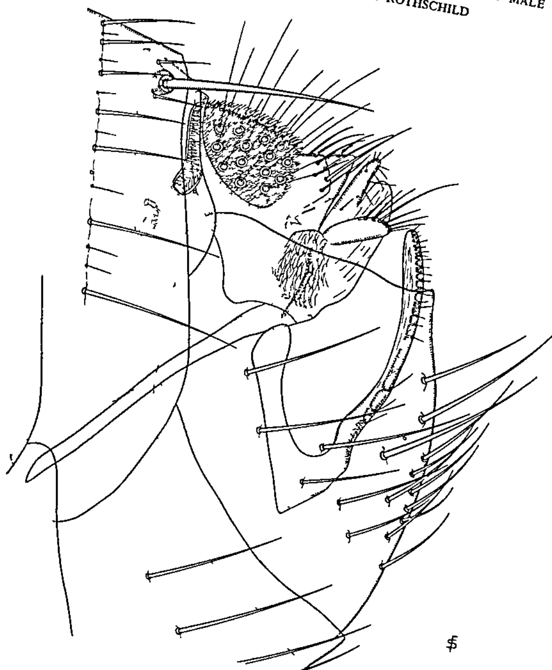


FIG 19 TERMINAL SEGMENTS AND GENITALIA OF FEMALE *XENOPSYLLA ASTIA* ROTHSCHILD

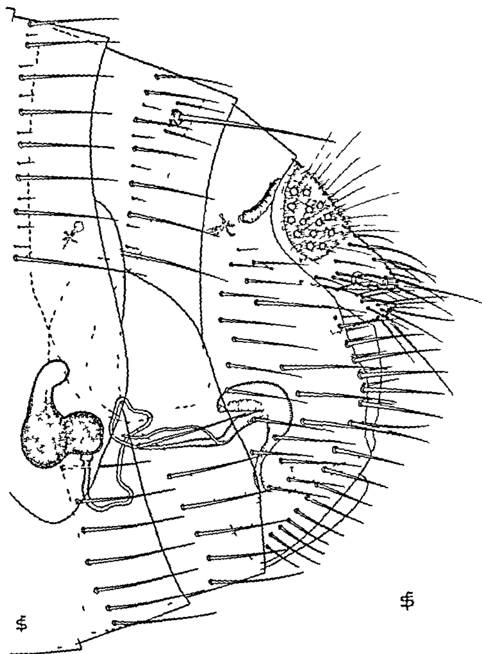
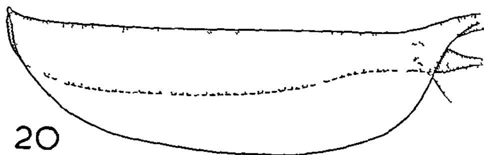
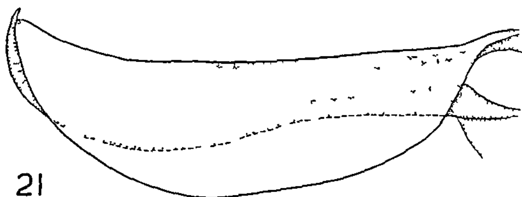
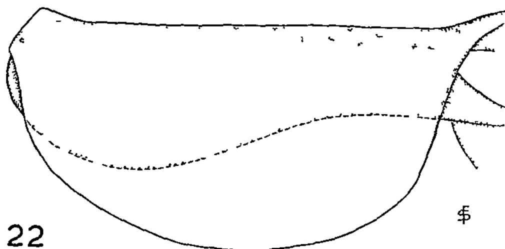


FIG 20 22 AEDEAGAL APODEME







20 Xenopsylla brasiliensis 21 Xenopsylla cheopis 22 Xenopsylla astia

FIG 23 HEAD PROTHORAX AND FORE COXA OF MALE NOSOPSYLLUS FASCIATUS (BOSC)

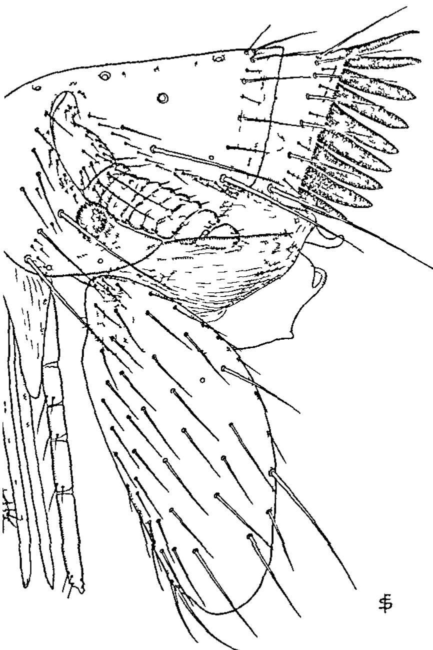
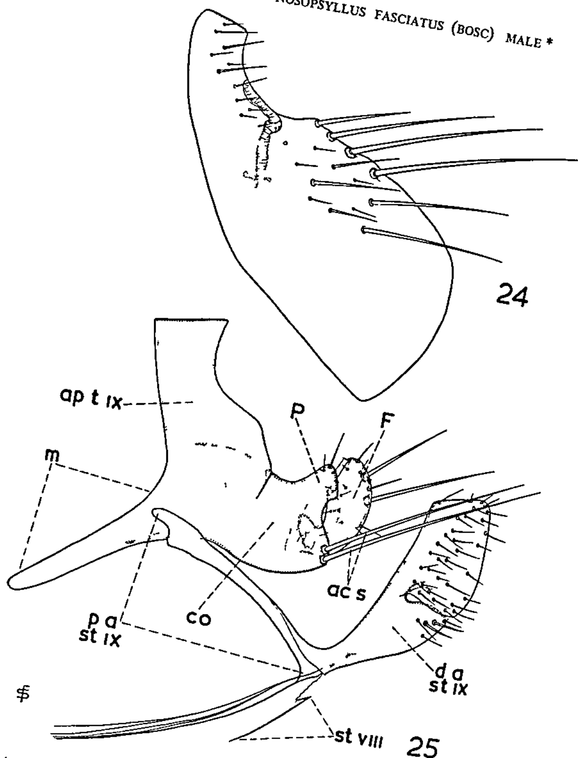


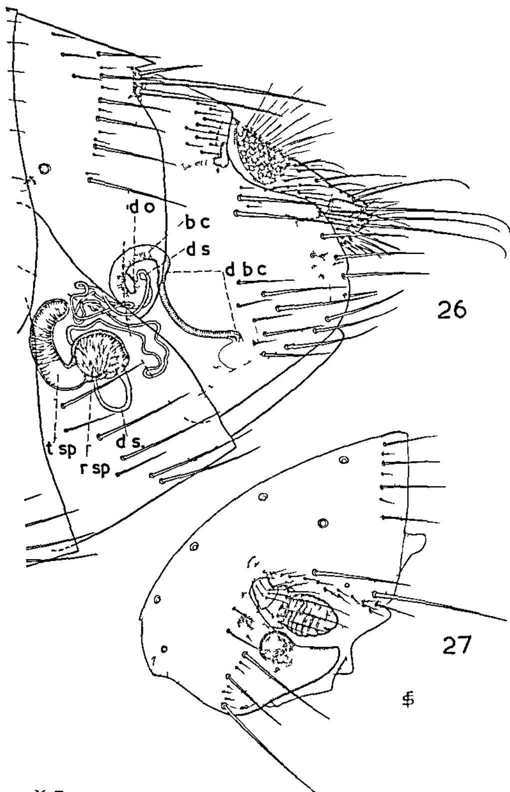
FIG 24 25 NOSOPSYLLUS FASCIATUS (BOSC) MALE *



24 Tergum VIII 25 Modified abdominal segment IX and sternum VIII

* *For explanation of lettering see page 650*

FIG 26 27 NOSOPSYLLUS FASCIATUS (BOSC) FEMALE *



26 Terminal segments and genitalia 27 Head

* *For explanation of letterings see page 650*

FIG 28 MODIFIED ABDOMINAL SEGMENT IX AND STERNUM VIII OF MALE MONOPSYLLUS ANISUS (ROTHSCHILD)

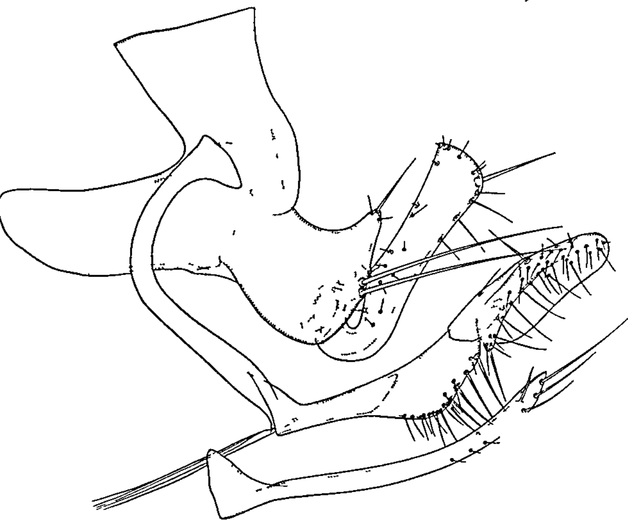


FIG 29 TERMINAL SEGMENTS AND GENITALIA OF FEMALE MONOPSYLLUS ANISUS (ROTHSCHILD)

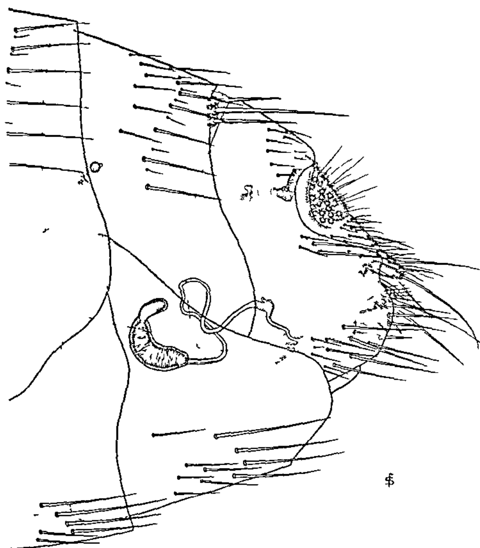


FIG 30 TERMINAL SEGMENTS AND GENITALIA OF MALE LEPTOPSYLLA SEGNIS (SCHONHERR)

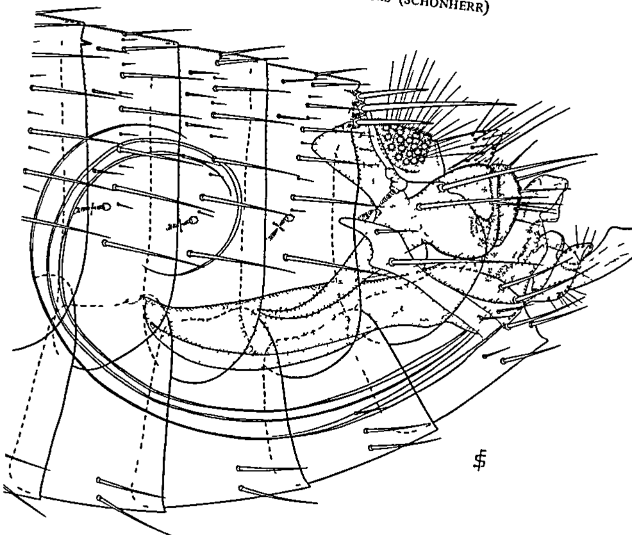


FIG. 31 TERMINAL SEGMENTS AND GENITALIA OF FEMALE LEPTOPSYLLA SEGNIS (SCHÖNHERR)

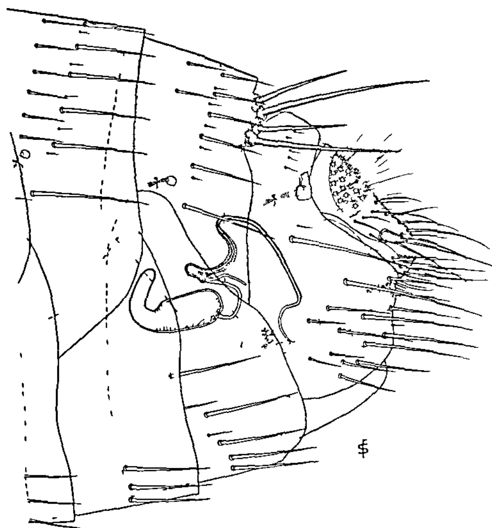
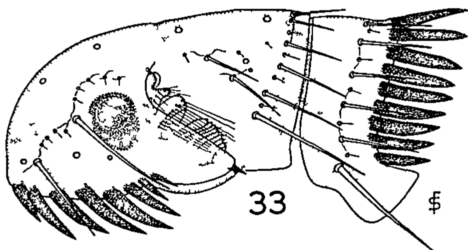


FIG 32 LEPTOPSYLLA SEGNIS (SCHÒNHERR)

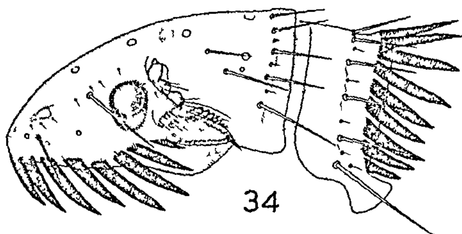
FIG 33 CTENOCEPHALIDES CANIS (CURTIS)

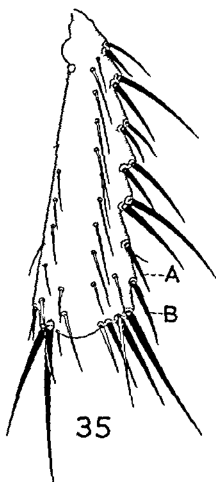
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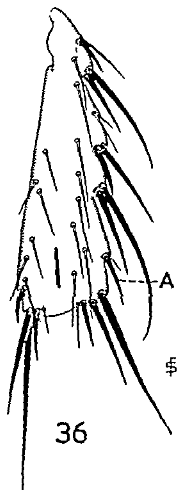


32 Head and prothorax ♂   33

FIG 34 CTENOCEPHALIDES FELIS FELIS (BOUCHE)
FIG 35 CTENOCEPHALIDES CANIS (CURTIS)
FIG 36 CTENOCEPHALIDES FELIS FELIS (BOUCHE)



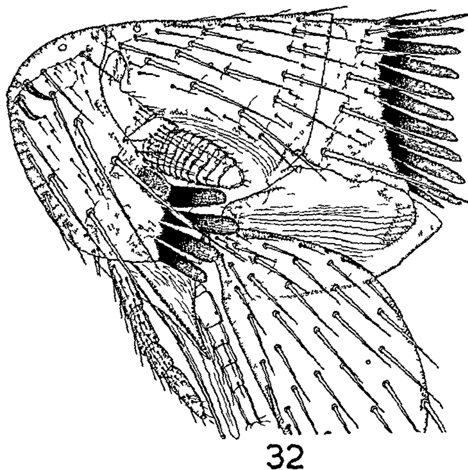




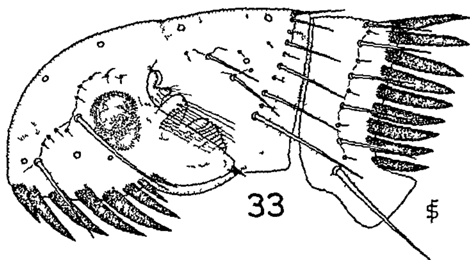
34 Head and pronotum ♀ 35 Hind tibia ♀ 36 Hind tibia

FIG 32 LEPTOPSYLLA SEGNIS (SCHONHERR)

FIG 33 CTENOCEPHALIDES CANIS (CURTIS)



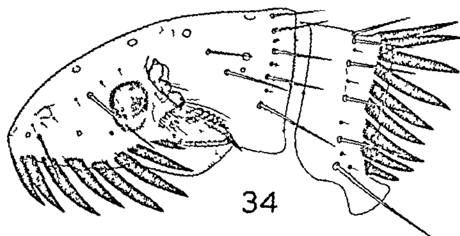
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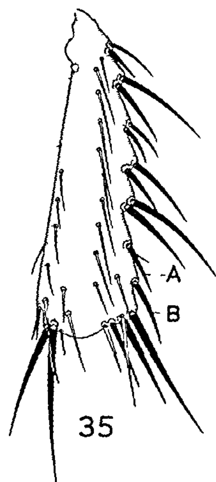


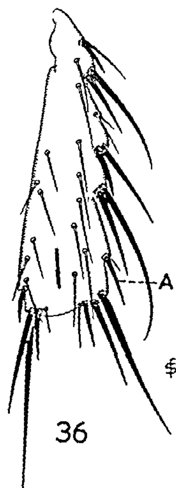
33

32 *Head and prothorax,* ♂ 33 *Head and pronotum* ♀

FIG 34 CTENOCEPHALIDES FELIS FELIS (BOUCHE)
FIG 35 CTENOCEPHALIDES CANIS (CURTIS)
FIG 36 CTENOCEPHALIDES FELIS FELIS (BOUCHE)





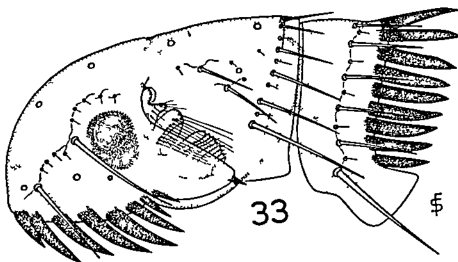


34 Head and pronotum ♀ *35 Hind tibia* ♀ *36 Hind tibia* ♀

FIG 32 LEPTOPSYLLA SEGNIS (SCHONHERR)

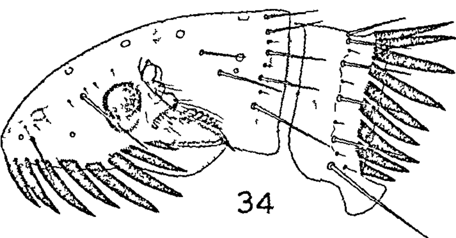
FIG 33 CTENOCEPHALIDES CANIS (CURTIS)

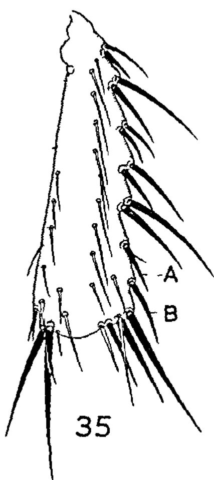
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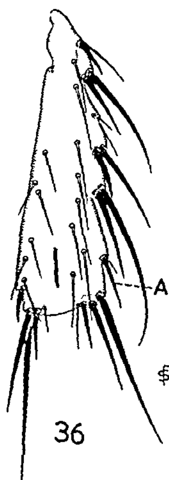


32 Head and prothorax ♂ *33 Head and pronotum* ♀

FIG 34 CTENOCEPHALIDES FELIS FELIS (BOUCHE)
FIG 35 CTENOCEPHALIDES CANIS (CURTIS)
FIG 36 CTENOCEPHALIDES FELIS FELIS (BOUCHE)

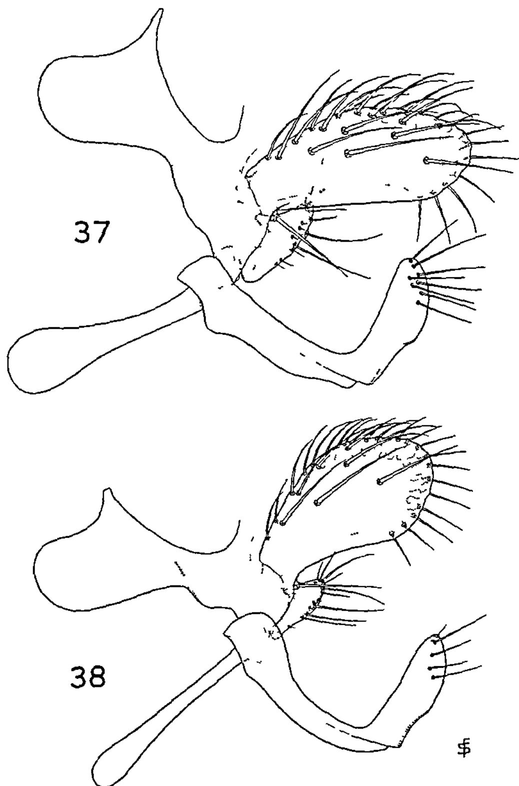






34 Head and pronotum ♀ 35 Hind tibia ♀ 36 Hind tibia ♀

FIG 37-38 MODIFIED ABDOMINAL SEGMENT IX OF MALE



37 Ctenocephalides canis (Curtis) *38 Ctenocephalides felis felis (Bouche)*

C 39 TERMINAL SEGMENTS AND GENITALIA OF FEMALE
CTENOCEPHALIDES CANIS (CURTIS)

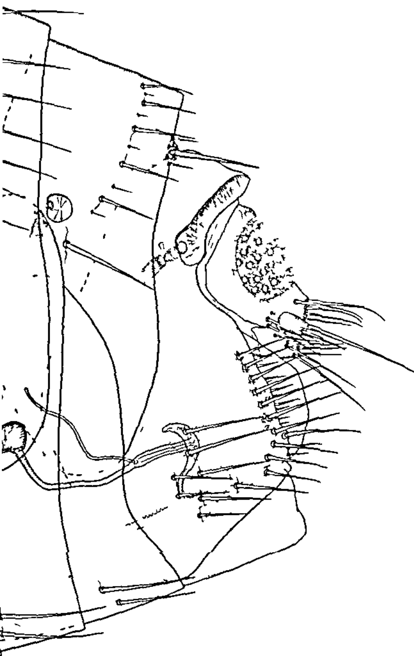
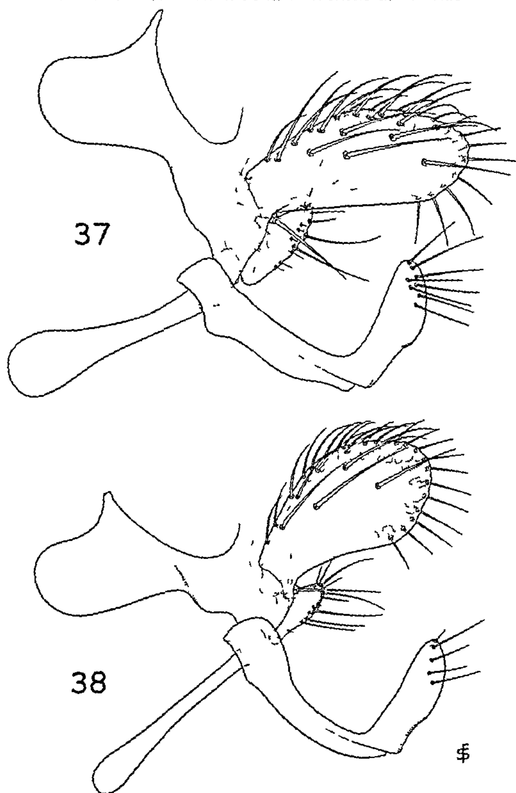


FIG 37 38 MODIFIED ABDOMINAL SEGMENT IX OF MALE



37 *Ctenocephalides canis (Curtis)* 38 *Ctenocephalides felis felis (Bouché)*

FIG. 79 TERMINAL SEGMENTS AND GENITALIA OF FEMALE CTENOCEPHALIDES CANIS (CURTIS)

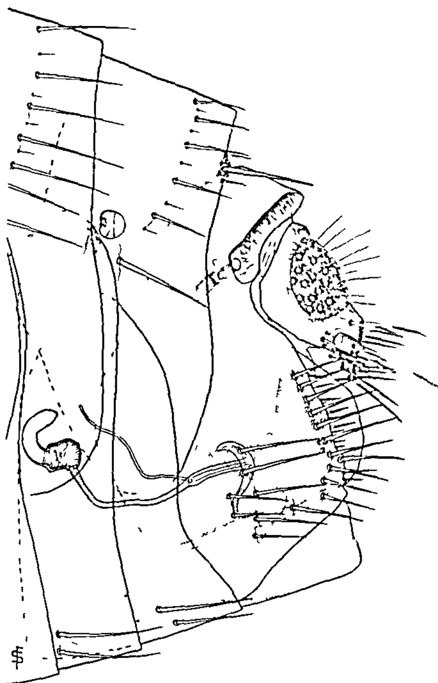
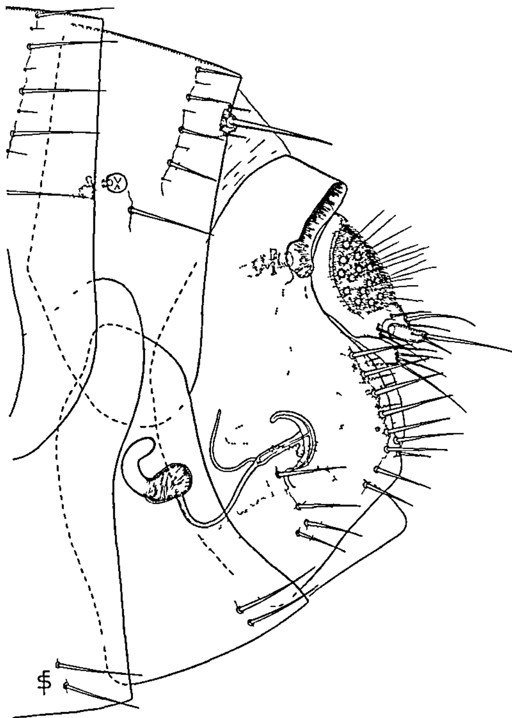


FIG 40 TERMINAL SEGMENTS AND GENITALIA OF FEMALE CTENOCEPHALIDES FELIS FELIS (BOUCHE)



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